CANCER METASTASIS: EXPERIMENTAL APPROACHES, THEORETICAL CONCEPTS, AND IMPACTS FOR TREATMENT STRATEGIES

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I. Introduction

One of the most urgent problems in the management of cancer is the prevention of metastasis formation. The ability of malignant cells to disseminate from a locally growing tumor and to form secondary lesions at near or distant sites is the most life-threatening aspect of cancer, and yet there are no good tools available for either diagnosis or treatment of metastases.

Until recently, radical cancer surgery was designed to ablate the primary tumor and its lymphatic drainage in the hope that dissemination would thus be prevented. However, in recent years this has been more critically evalu-

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ated, although survery has improved technically to such an extent that even the most horrendous primary lesions can be quite successfully removed. Cancer surgeons are becoming aware that metastases are their main enemy and that a small number of disseminated cells could later give rise to secondary deposits that might defeat their efforts and kill the patient.

In the past, the choice of cancer treatment has been almost entirely a matter of the physician's personal opinion. There have rarely been sound scientific evaluations of the costs and the benefits to the patient. Meanwhile, as will be described, new scientific insight into the metastatic process is beginning to provide the kind of information necessary for an evaluation of therapeutic strategies.

It is well known from clinical cancer that the most rapidly growing tumors are usually the most capable of producing local or distant metastasis (Sugarbaker, 1979, 1981). For example, children with Burkitt's lymphoma have fast-growing tumor lesions which generate aggressive metastases and the patients succumb rather quickly. On the other hand, there are certain types of cancer such as basal cell carcinoma in which lesions are highly invasive but rarely metastatic. Another important aspect is the relationship between primary tumor size and incidence of metastasis. In general, the incidence of metastasis appears to increase statistically with increased tumor size, although the same type of cancer often produces quite diverse survival patterns among individual patients (Willis, 1973). There are, however, examples of rapidly spreading tumors in which there is little relationship between primary tumor size and incidence of metastasis such as small cell carcinoma of the lung.

The locations of most initial metastases, at least of cancers with moderate metastatic potential (Sugarbaker, 1981), appear to be determined simply by regional anatomy (Ewing, 1928). Human cancer cells frequently travel from the primary lesion to regional nodes and from nodes to veins via lymphaticovenous communications (Weiss, 1976; Gilbert *et al.*, 1980). However, in approximately 20% of patients with carcinomas that eventually metastasize to lung, liver, bone, or brain, no lymph node involvement can be detected clinically or histologically. About 90% of women with lymph node negative breast cancer will survive for 5 years or more after adequate local therapy, but there are still 10% who relapse. Similarly, about 60% of patients with extensive lymph node involvement in this disease will be dead within 5 years, whereas another 40% will remain well. These figures illustrate the level of prognostic significance of regional lymph node involvement. It is obvious that we need better markers for tumor prognosis. Also, we need better understanding of the pathways of metastases and their sequence of development with time based on a clear and coherent scientific concept.

Spread and growth of cancer cells seem to depend to a great extent upon the venous pathways involved. Tumors rarely invade arteries larger than

precapillary arterioles. The arterial circulation seems to be a particularly hostile environment for tumor cells, which is best exemplified by the rarity of arterial dissemination. Muscle, kidney, spleen, intestine, skin, and heart are involved in less than 10% of all metastases, although the arterial output to these organs is equivalent to about 70% of the total arterial output. In contrast, metastasis in the bone, a tissue that receives less than 5% of the total arterial output, is quite common. Of interest also is the difference between bone, which is a fairly common site of metastases, and cartilage, which is hardly ever involved (Eisenstein *et al.*, 1975).

The most frequent organ site of distant metastasis in many types of cancer appears to be the first organ encountered by circulating tumor cells. However, there are a number of examples of blood-borne clinical metastasis not explainable by lodgement in the first capillary system encountered (Sugarbaker, 1981). Reasons for this organ site predilection will be discussed later.

Due to the potential of metastatic spread, cancer—at least from a certain stage on—must be considered as a generalized disease and thus cannot be treated only locally. It is a basic misconception of strategy if physicians try to treat a generalized disease with localized therapy. There is a growing awareness that new approaches to the treatment of cancer are needed and that these must be based upon an increased understanding of the pathogenesis of metastasis.

New developments in gene technology, cell, tissue, and organ culture under defined conditions, as well as progress in cancer chemotherapy, hormone therapy, and immunotherapy, have given rise to considerable opportunities to investigate the biology of cancer cells and the effects of a large number of substances on their behavior. Considerable attention is now being focused on the most crucial processes that differentiate benign from malignant growth, namely, invasion, dissemination, and metastasis formation. A dramatic increase in the past few years of research in clinical and experimental metastasis has led to the foundation of several new journals which specialize on this subject, namely (1) Invasion and Metastasis (Karger, since 1981) (2) Cancer Metastasis Reviews, (Martinus Nijhoff Publishers, The Netherlands, since 1982), and (3) Clinical and Experimental Metastasis (Taylor and Francis Ltd., London, since 1982).

The complexity of the phenomenon of metastasis is reflected in the diversity of disciplines engaged, which range from biophysics to clinical oncology and involve cell biologists, biophysicists, biochemists, immunologists, physicians, etc. Because of the complexity of the field and because research is being done by so many academic specialties, investigators often find it difficult to keep up with all of the developments, to interpret individual findings, or to develop concepts consistent with the information obtained from many different disciplines.

It is therefore the aim of this article to familiarize those interested in the

subject with (1) important issues and questions that are being addressed at present, (2) new approaches developed to study such questions, and (3) new results and concepts developed. A previous article in this series on the biology of cancer invasion and mestastasis (Fidler *et al.*, 1978) is recommended as a basis.

II. Metastasis Research

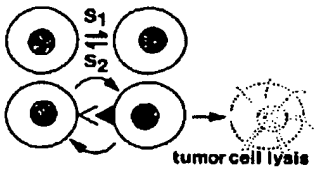
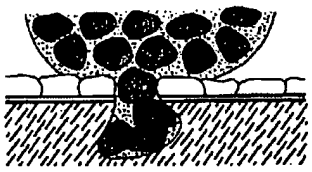
A. Overview: From Oncogenes to Metastases

Table I illustrates the different levels of research in the field of cancer metastasis. The table also indicates the kind of questions investigated at each level and the experimental approaches used.

Recent advances in molecular biology have led to the identification of oncogenes as general and important determinants of carcinogenesis. Oncogenes can be defined as eukaryotic genes that have been conserved in evolution and therefore presumably fulfill an essential function in the cell. They code for a protein and have the potential to act as dominant genetic trait, for instance, after transfer into a normal cell environment. Although oncogenes were originally defined as transforming genes in retroviruses, incorporation into a virus vector is not an essential criterion of an oncogene. Oncogenes can become abnormally expressed by a variety of mechanisms (mutational change, promoter insertion, change in transcriptional or in translational control, chromosomal rearrangement with oncogene translocation, etc.). Recently there have been reports also of concerted actions of several oncogenes. Whether oncogenes might also play a special role in tumor progression and in metastasis is unclear at present. In any case, the new techniques of molecular biology have enabled an approach to metastasis-related questions at the molecular level.

What happens after oncogene activiation within a single cell of a multicellular organism is less clearly established. The misinformation carried by the cell must be reproduced several million times by clonal outgrowth before it can be clinically detectable. This, among other reasons, may explain the delay time or latent period. Failure of feedback tissue control systems and perhaps concomitant failures of host defense systems that may normally safeguard against this process may be involved. This level of cellular interaction is more difficult to study in precancerous stages than it is in overt cancer and in metastasis. Metastasis research at the level of cellular interaction has been performed *in vitro* and *in vivo* to study tumor subpopulation interactions, metabolic cooperation, immune cell–tumor cell interactions, and organ cell–tumor cell interactions.

TABLE I
CANCER METASTASIS RESEARCH AT DIFFERENT LEVELS

Level of research	Questions studied	Experimental approach
Single cell 	Role of onc genes Role of cell surface molecules Role of state of differentiation	DNA transfection Effect of genetic manipulation Effects of DNA demethylation
Cellular interactions 	Tumor subpopulation interaction Metabolic cooperation Immune cell–tumor cell interactions Organ cell–tumor cell interactions	Comparison of clones and mixed cultures Test for drug sensitivity Cytotoxicity test Cytostasis test Immune escape mechanisms Adhesion and inhibition tests
Tissue interactions 	Mechanisms of angiogenesis Mechanism of invasion Regulatory effects from tissue microenvironment	*In vitro* studies using cultures of tissue fragments or biological membranes Signal perception and tumor cell response
Whole organism 	Mechanism of metastasis Mechanism of host resistance Improvements of cancer therapy	Comparison of selected subpopulations Immunological studies Monoclonal antibodies Monoclonal T cells Immunotoxins Drug-targeted liposomes Lymphokines

Tumor cell–tissue interactions are the next higher level of complexity in metastasis research. There are several critical steps of tissue interactions that probably determine whether the transformed clone will or will not remain under control of the host. One such critical point is the evolution of a blood supply for the clone. Without a special nutrient supply via angiogenesis, the

size of the clone will be strictly limited. Angiogenesis may also be taken as a starting point for hematogenous dissemination and visceral metastasis formation.

Mechanisms of cancer metastasis formation, host resistance phenomena, and cancer therapy also have to be studied at the level of the whole organism. These important subjects require careful animal experimentation since even the most sophisticated *in vitro* system cannot mimic properly the situation within an intact organism. Immune defense reactions or cytotoxic anticancer drugs may be very effective outside the body. Their relative effectiveness for cancer therapy has to be tested, however, *in vivo* in terms of prolongation of disease-free interval, reduction in number of metastases, or prolongation of life expectancy.

All three kinds of control on the cell in a normal location are probably less effective at the metastatic site. The original genetic damage has diminished internal control of the cell; the transplantation to a new site has reduced the local control by adjacent cells, matrix, and soluble factors; and finally the host defense system for external control is deteriorating with further disease progression.

B. Animal Models

Studies on human metastatic tumor cells have been hindered mostly by the lack of appropriate *in vivo* assay systems. An exception is the use of immunosuppressed animals, the most popular of which is the congenitally athymic nude mouse. There are a number of inherent problems, however, associated with such a human tumor xenograft model (Sordat and Wang, 1984). Since the nude mouse has a high level of natural defense mechanisms [for instance, natural killer (NK) cells and macrophages], there may be highly selective pressures exerted on the human tumor material so that the tumors that eventually arise may not represent the same cellular diversity as the original tumor. In addition, there may be particular tissue stroma and endocrine requirements for growth and spread of human tumors that may not be provided optimally in the foreign host. Another problem is the lack of comparable lines from both primary and metastatic lesions of the same patient, which is probably due to the difficulties encountered in obtaining fresh tissues immediately after surgical excision and the lack of reproducible methods for routine cultivation of human tumor cells.

Investigations on the mechanism of cancer metastasis have therefore mostly depended on animal model systems, most of which represent rodent tumors transplanted in their syngeneic hosts. There are obvious and serious limitations with attempts to compare animal data from such tumor systems with human cancer because the models do not accurately reflect or mimic

some of the events that occur when a spontaneous human neoplasm progresses and metastasizes in its host. Nevertheless, the extensive use of transplantable animal tumors that share a common genetic background with their host but differ in their metastatic phenotypes and cell properties has provided most of our current knowledge about tumor and host characteristics in metastasis.

The available animal tumor models for studying metastasis and their respective limitations as well as advantages have been summarized and discussed in detail elsewhere (Fidler *et al.*, 1978; Nicolson, 1982; Poste and Nicolson, 1983). Table II lists some representative examples of such tumor models, all of which consist of subpopulations with different metastatic properties.

B16 melanoma and Lewis lung carcinoma, which have been used most extensively in metastasis research, are of spontaneous origin (both from the C57BL/6 mouse) but have been propagated *in vivo* and *in vitro* for almost 30 years, which is approximately 15 times the average life span of their original host. Such tumors are highly selected for rapid growth and do not mimic the slowly growing human or animal primary tumors, which often take years to reach the size at which they are diagnosed and treated. Most of the long-transplanted animal cell lines are extremely anaplastic and can be considered as models only for rapidly growing, very aggressive human tumors.

Newly induced tumors, such as chemically or virus-induced neoplasms, are seldom metastatic. Many spontaneous tumors are capable of metastasizing widely, but their unpredictable occurrence makes them difficult to work with and to obtain reproducible results. There are some kinds of spontaneous tumors that arise at relatively high incidence in certain strains of laboratory animals and in domestic or farm animals, such as mammary carcinomas, osteogenic sarcomas, and lymphomas in dogs and cats (Hewitt, 1978). The use of such tumors from larger nonrodent animals for experimental studies, however, is limited because of limitations in space, time, and money and also because most such animals are not sufficiently inbred to allow their use as recipients for tumor transplantation.

C. Important Issues and Questions

The choice of an animal tumor system depends most of all on the question to be studied. In any case, the origin and subsequent passage history of the tumor to be studied should be known before it is received in the investigator's laboratory in order to avoid such artifacts as Hewitt *et al.* (1976) pointed out and criticized.

Important questions that are presently under investigation in model systems can be formulated as follows:

TABLE II

EXAMPLES OF ANIMAL TUMOR MODELS WITH SUBPOPULATIONS DIFFERING IN METASTATIC PROPERTIES

Species	Tumor	Subpopulation	Major sites of metastases	References
Mouse	B16 melanoma	Clones	Lung>other sites	Fidler and Kripke (1977)
Mouse	B16 melanoma	Organ-selected variants	Ovary, brain	Brunson *et al.* (1978), Brunson and Nicolson (1979)
Mouse	B16 melanoma	Immunoresistant variants		Fidler *et al.* (1976)
Mouse	B16 melanoma	Invasion selected	Lung, lymph nodes	Poste *et al.* (1980)
Mouse	B16 melanoma	Lectin resistant		Tao and Burger (1977)
Mouse	3LL Lewis lung carcinoma	Lung selected	Lung	Fogel *et al.* (1979)
Mouse	3LL Lewis lung carcinoma	Clones varying in H-2		Eisenbach *et al.* (1983)
Mouse	Mammary carcinoma	Sublines		Heppner *et al.* (1978)
Mouse	UV 2237 fibrosarcoma	Clones	Lung	Kripke *et al.* (1978), Raz *et al.* (1981)
Mouse	MDAY-D2 undifferentiated	Lectin resistant, immunoselected	Liver, lung	Kerbel *et al.* (1982), Dennis *et al.* (1981b), Frost and Kerbel (1981)
Mouse	Eb/ESb lymphoma	CTL resistant	Liver, lung, spleen	Bosslet and Schirrmacher (1981), Schirrmacher *et al.* (1982c)
Mouse	Eb/ESb lymphoma	Plastic adherent		Fogel *et al.* (1983)
Mouse	Eb/ESb lymphoma	Spleen selected	Spleen	Cheingsong-Popov *et al.* (1983)
Mouse	SV 3T3 sarcoma			Nicolson *et al.* (1978)
Rat	ARG-1-RT7 hepatocarcinoma	Clones	Liver	Talmadge *et al.* (1979)
Chicken	HV-transf. AL.2 lymphoma		Liver	Shearman and Longenecker (1980)
Human	Adenocarcinoma	Ascites	Lung	Takahashi *et al.* (1978)

1. Is there a metastatic phenotype and if so, what are its cellular properties? Can this explain why some tumors metastasize and others do not?
2. How stable are tumor cell subpopulations or clones, and what is the origin of tumor heterogeneity?
3. Is metastasis a random, a selective, or an adaptive process? To what extent do these phenomena influence metastatic spread?
4. How can the organ preference of certain tumors be explained? How and at what levels is this phenomenon determined by tumor cell properties or by properties of the host?
5. What host influences are there in cancer metastasis? What is the role of the microenvironment (cells, extracellular matrix, soluble factors)? Can metastatic cells receive signals from the microenvironment? How do metastatic cells escape immunological host control?
6. How do some micrometastases remain dormant for prolonged periods of time? What makes them reactivate?
7. Does metastasis proceed in sequential steps? If so, is the whole process equivalent to the sum of the sequential steps or is it still more complex?
8. How does a growing primary tumor "condition" its host and what is the influence of the primary tumor on the growth of metastases?
9. Is metastasis a product of tumor–host interactions? If so, what is the mechanism of interaction between cancer cells and host tissues in sequential steps of the metastatic cascade, e.g., in angiogenesis, invasion, capillary adhesion, and immune escape. Can such processes be manipulated toward the advantage of the host?
10. What are the consequences of tumor heterogeneity, instability, and subpopulation interactions for cancer therapy?

The list of questions is not complete but it may give some insight into the area and may show the nature and fundamental importance of the issues studied. It should be kept in mind that without appropriate model systems and test methods, these questions cannot be investigated at all.

D. Assay Procedures and Selection of Metastatic Subpopulations

Before illustrating recent progress in the field, I would like to discuss some methodological aspects, namely (1) assay procedures to test for metastatic capacity and (2) selection procedures to obtain tumor subpopulations of different metastatic capacities. To test for metastatic capacity, transplantable tumors are inoculated locally (subcutaneously or intramuscularly) and then the formation of "spontaneous" metastases in various major organs is moni-

tored. In contrast to this spontaneous metastasis assay, in the "experimental" metastasis assay tumor cells are injected directly into the circulation, mostly by tail vein inoculation. The iv method circumvents the initial steps of metastasis and tests mostly for capacity to survive in the circulation and to implant in internal organs. This implantation phase includes arrest in capillaries, extravasation, and growth. Although tumor cells may successfully colonize organs after iv inoculation, they may have low spontaneous metastatic potential because of low rates of entry into the circulation after local inoculation. Welch *et al.* (1983) claim that there is good agreement between experimental and spontaneous metastasis assays, whereas others (Stackpole, 1981) reported that this was not the case. Both the spontaneous and the experimental metastasis assays have been reviewed and discussed critically (Hagmar *et al.*, 1983).

Since the interaction of tumor cells with their environment is mediated by cell surface constituents, surface components are thought to play a major role in metastasis. Figure 1 illustrates two basically different approaches to correlate metastatic capacity with tumor cell surface properties (Burger, 1980). Both *in vivo* selection of metastatic variants and testing for cell surface properties and *in vitro* selection of cell surface variants and testing for metastatic properties have been used in these studies.

In vivo sequential selections for metastatic subpopulations were already being performed several decades ago (Klein, 1955). They became popular when Fidler exploited this procedure more systematically in a series of elegant experiments. He started with sequential selections to enrich B16 melanoma cells for subpopulations with enhanced metastatic capacity or

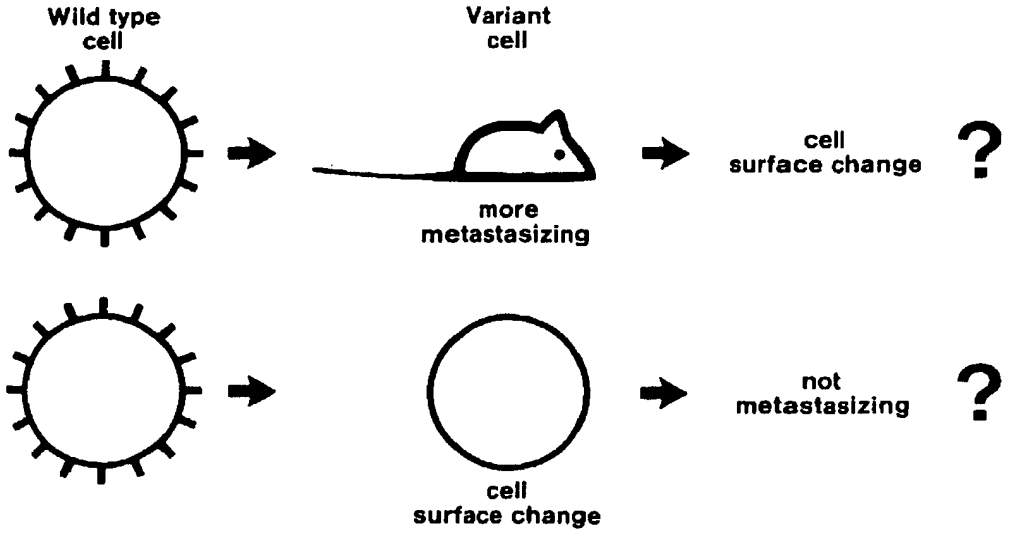


FIG. 1. Two different approaches to a search for correlations between alterations in metastatic capacity and cell surface alterations. (From Burger, 1980.)

with enhanced potential to colonize specific organs, such as brain, liver, or ovary (see Table II).

In vitro selection procedures have been employed to isolate tumor cell variants with altered cell surface properties such as increased resistance to plant lectin toxicity (Tao and Burger, 1977; Dennis and Kerbel, 1981), lymphocyte-mediated cytotoxicity (Fidler *et al.*, 1976), NK-cell-mediated killing (Hanna and Fidler, 1981), or antibody-complement-mediated lysis (Frost and Kerbel, 1981). Other procedures selected for changed adhesiveness to plastic (Fogel *et al.*, 1983), substratum (Briles and Kornfeld, 1978), or endothelial cells (Nicolson, 1982) or for increased ability to invade bladder tissue (Hart, 1979) or blood vessels (Poste *et al.*, 1980). When assayed *in vivo*, such *in vitro*-selected variants showed alterations in their metastatic behavior. Another *in vitro* procedure employed for obtaining metastatic tumor variants has been single cell cloning. Recent studies, however, have pointed out that clonal tumor cell populations are not always stable. In fact, in the majority of cases analyzed systematically, cloned tumor cell lines turned out to have an inherent instability. The evidence for this and the impact of this finding will be discussed below (Section IV).

In order to increase the frequency of variant generation, some investigators mutagenize their tumor lines, whereas others try to avoid the use of drugs because of their multiple effects and concentrate on spontaneous variants. Boon and associates (Boon and Kellermann, 1977; Boon, 1983) and recently also Frost *et al.* (1983) reported that mutagenesis of normally tumorigenic tumor cell lines followed by cloning of the surviving cells often gives rise to a very high frequency (10–90%) of clones which are unable to grow progressively in normal syngeneic mice. Such clones usually have acquired new strong tumor antigens that lead to their rejection in immunocompetent but not in immune incompetent mice. Similar results were recently obtained with the nonmutagenic drug 5-azacytidine, which causes undermethylation of DNA and deregulation of gene expression (Frost *et al.*, 1984). These findings suggest that the high frequency of tumor variants was not generated by classical mutation events but rather by "epigenetic" mechanisms. The altered methylation patterns can be somatically inherited, although not with perfect fidelity (Wigler *et al.*, 1981), thereby raising the distinct possibility that "methylation changes can masquerade as mutations" (Riggs and Jones, 1983). While the majority of clones derived from 5-azacytidine-treated tumor cell cultures had an increased immunogenicity, there have also been clones isolated which showed increased metastatic capacity. These studies have important implications for our understanding of tumor immunogenicity and of tumor variant generation and will therefore be discussed again in the context of mechanisms of tumor progression (Section IV).

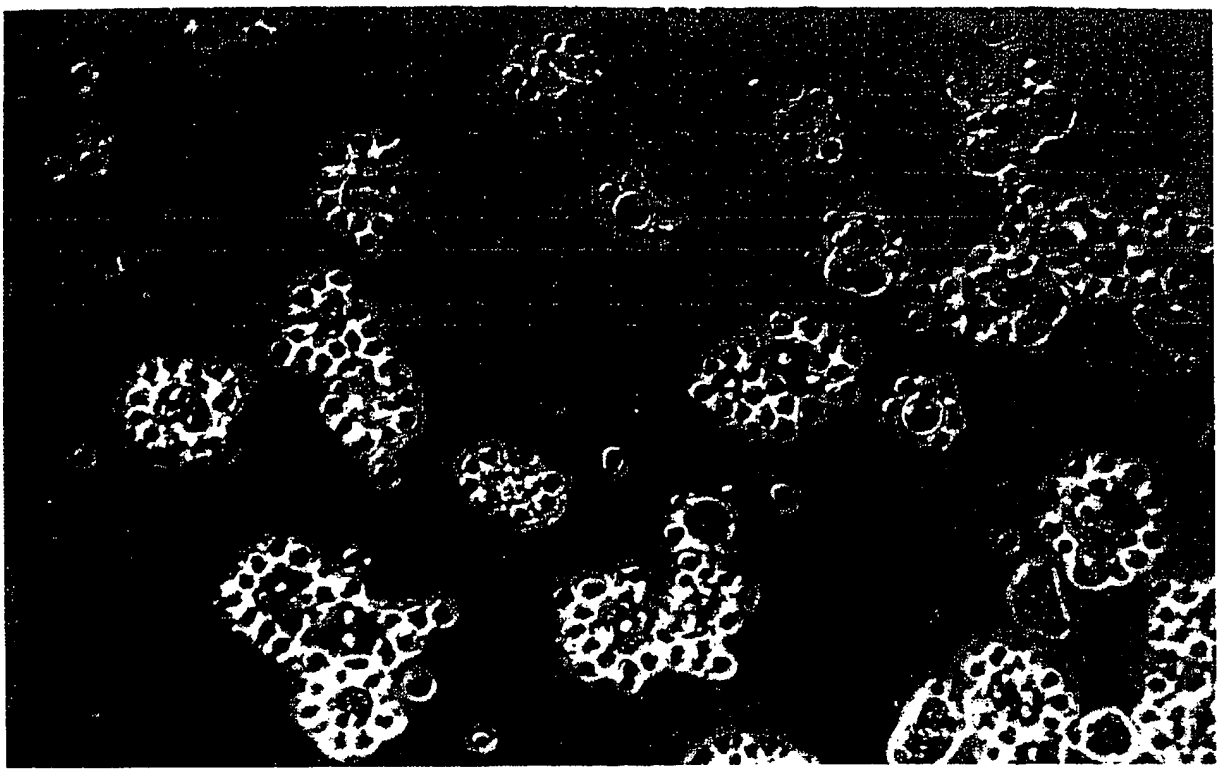
E. Cell Surface Properties and Cancer Metastasis

Some of the findings derived from experimental studies comparing selected subpopulations with different metastatic properties provided strong evidence for the importance of cell surface properties in cancer metastasis. These are summarized in Table III. The evidence comes from both types of approaches of Fig. 1 as well as from recent inhibition studies with monoclonal antibodies directed against important cell surface molecules. Several authors (Rapin and Burger, 1974; Chatterjee and Kim, 1977, 1978; Irimura *et al.*, 1981) have suggested that cell surface carbohydrates are important in cancer metastasis. Yogeeswaran and Salk (1981) found that tumor lines with high metastatic capacity showed increased levels of sialylation of cell surface glycoconjugates when compared with related lines of low metastatic potential. We have suggested recently (Schirrmacher *et al.*, 1982b) that not only the amount but also the specific positioning of sialic acid at the cell surface may be important: Via changes in positioning, sialic acid could lead to either blocking or unblocking of cellular adhesion sites or antigenic determinants and could thus influence metastatic capacity.

Lectin–carbohydrate interactions have been found to play a crucial role in many intercellular recognition processes. We recently provided the first molecular description of such an interaction between organ-derived normal

TABLE III
Evidence that Tumor Cell Surface Properties Are Important for Metastatic Capacity

Evidence	Reference
Tumor wild type and metastatic variant show cell surface change	
Changes in glycoproteins and glycolipids	For review see Nicolson (1982)
Changes in lectin-binding characteristics	For review see Schirrmacher *et al.* (1982)
Tumor wild type and surface variants show change in metastatic capacity	
Wheat germ agglutinin-resistant variants and metastatic revertants thereof	Tao and Burger (1977), Kerbel *et al.* (1982), Finne *et al.* (1980), Dennis and Kerbel (1981)
GP 70 cell surface variant	Reading *et al.* (1980)
Effects of tunicamycin	Irimura *et al.* (1981)
Effects of fusion of membrane vesicles	Poste and Nicolson (1980)
Antibodies against distinct cell surface components can have inhibitory effects on metastasis formation	Nicolson (1982), Vollmers and Birchmeier (1983a,b), Vollmers *et al.* (1984)



Tumor line	Pretreatment	% hepatocytes with tumor cell rosettes
Eb	–	5
Eb	neuraminidase	89
Eb	β-galactosidase	0
ESb	–	74
ESb	neuraminidase	92
ESb	β-galactosidase	26

FIG. 2. Rosette formation between hepatocytes (central cells) and liver-metastasizing ESb tumor cells. (Reproduced from Schirrmacher *et al.*, 1982.)

parenchymal cells—hepatocytes—and liver-metastasizing tumor cells (Schirrmacher *et al.*, 1980; Cheingsong-Popov *et al.*, 1983). Figure 2 shows this interaction in the form of rosettes between hepatocytes and highly metastatic ESb tumor cells. Removal of β-galactosyl groups from the ESb tumor cells by β-galactosidase treatment resulted in a reduction of spontaneous liver-rosette-forming capacity. The low metastatic parental type Eb cells had only a low liver cell rosette-forming capacity unless the cells were pretreated with neuraminidase. This treatment leads to exposure of free β-

galactosyl residues. The hepatocytes were found to bind ESb tumor cells through lectin-like hepatic binding proteins (HBPs) with molecular weights of 52, 56, and 110 kDa and specificity for D-galactosyl and *N*-acetyl-D-galactosaminyl residues (Cheingsong-Popov *et al.*, 1983). Thomsen-Friedenreich (T) antigen, which expressed immunodominant Galβ1-3GalNac determinants, was found to be present on our tumor cells (Springer *et al.*, 1983a). In soluble form, this T antigen was a powerful inhibitor of the spontaneous hepatocyte rosettes (Springer *et al.*, 1983b). More than 10 different cell surface glycoproteins of ESb tumor cells and none of Eb-type tumor cells served as ligands in the hepatocyte adhesion. A possible relevance of such an interaction for the organotropism of cancer metastasis was suggested from the finding that spleen-selected ESb sublines differed from liver-selected ones in their organotropism as well as in their ability to form hepatocyte rosettes (Cheingsong-Popov *et al.*, 1983).

From these and other findings (Altevogt *et al.*, 1983; Fogel *et al.*, 1983) we recently formulated a new hypothesis that underlines the importance of site-specific positioning of sialic acid (Schirrmacher *et al.*, 1982b). We propose (1) that cell surface carbohydrate changes take place during tumor progression; (2) that selection favors changes that are not random but rather cell surface site specific, and (3) that glycosyltransferases may play an important role in these changes, leading to the masking of some sites (lectin receptors, adhesion sites, antigens) and exposure of others (other lectin receptors, adhesion sites, or antigens).

III. Cascade Theory of Metastasis

A. Clinical Data

Clinical autopsy data on visceral tumor metastases have been analyzed with respect to incidence and sequence in order to delineate pathways of metastasis. In one study more than 4700 consecutive autopsies were analyzed, first with standard statistical methods ("analysis of variance") (Bross and Blumenson, 1976) and later by focusing on specific primary sites using the "cascade analysis" as a new statistical tool (Viadana *et al.*, 1978a,b). The main purpose was to provide an objective procedure for determining patterns in the sequence of events of the metastatic process. This analysis together with others led to the foundation of the cascade theory of metastasis. It clearly suggested that the process of metastasis takes place step by step and involves both systematic processes of dissemination and chance mechanisms. In the terminology of biostatistics, it is a *sequential* as well as a *stochastic* process. Experimental animal data in addition suggest that the

metastatic process is also a *selective* one (Poste and Fidler, 1980). The extent to which cancer metastasis is selective or random seems to depend on the parent tumor population (Talmadge and Fidler, 1982).

Figure 3 illustrates major pathways of metastases from two clinically important types of cancer, namely, carcinoma of the lung and of colon. Lung cancer can disseminate via the left heart ventricle directly into the arterial circulation resulting in an arterial pattern of spread. Colon cancer tends to metastasize via the mesenteric lymphatics and the portal venous system into the liver as the first generalizing site. From there the cells can disseminate further via the right heart ventricle to the lung. Most types of cancer show a lymphaticovenous pattern of spread, like colon carcinoma. Major pathways of metastases of important types of human cancer can be summarized as follows (Gilbert *et al.*, 1980):

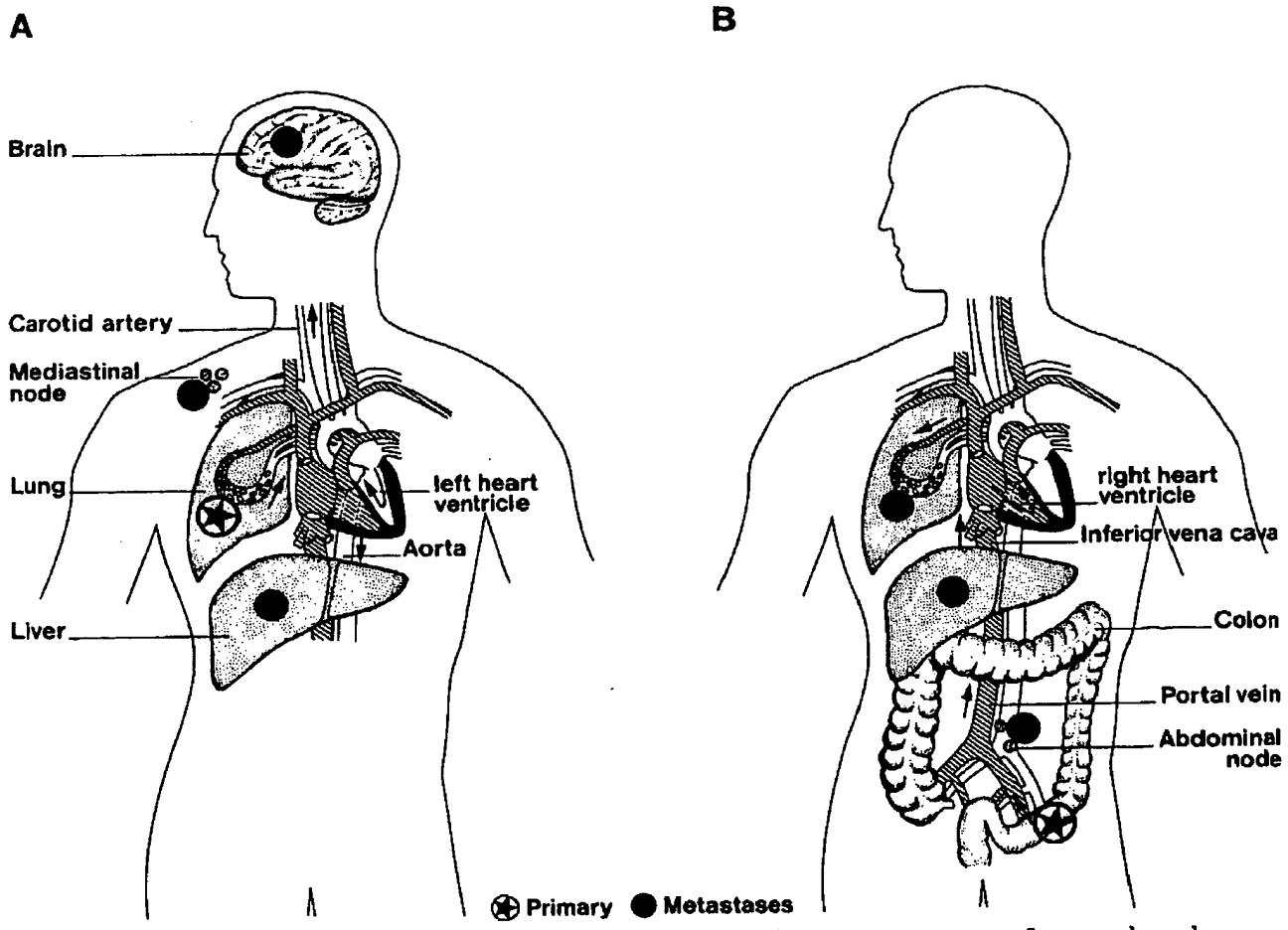


FIG. 3. Major pathways of metastasis from two clinically important types of cancer based on Gilbert *et al.* (1980). Notice the difference between lung cancer (A), which can disseminate via the left heart ventricle directly into the arterial circulation resulting in an arterial pattern of spread, and colon cancer (B), which tends to metastasize via the mesenteric lymphatics and the portal venous system into the liver as the first generalizing site.

1. Head and neck cancers metastasize to the lymphatics and remain confined in this area. Nasopharyngeal cancer tends to go to the liver and bone more frequently than other head and neck cancers. The spread to bone is via Batson's plexus, a slow-moving valveless system of paravertebral veins.

2. Breast cancer metastasizes either directly to the lung via the lymph nodes and superior vena cava or passes directly to the bony skeleton via the paravertebral veins.

3. Lung cancer is the only tumor that has direct access to the arterial circulation via the pulmonary vein and the left heart ventricle. It can thus spread widely to many organs including the brain (see Fig. 3).

4. Colorectal carcinomas tend to metastasize via the mesenteric lymphatics and portal venous system into the liver as the initial resting place. From the liver these tumors can metastasize to the lungs via the inferior vena cava, the right ventricle, and the pulmonary artery.

5. Tumors of the testicle metastasize via the lymphatics to nodes of the periaortic area and then enter the subclavian veins to go to the right heart and finally to the lungs. Liver metastases occur late in the disease.

6. Ovarian cancer remains confined for long periods of time in the highly favorable environment of the abdominal cavity, especially the peritoneal surfaces, the posterior gutters, and the diaphragm. These tumors invade the liver only in a small percentage of cases at a very late stage, usually by direct invasion from omental disease or mesenteric venous emboli from omental implants. Lung metastases also occur, but late in the disease.

7. Gynecologic tumors tend to involve primarily lymphatic structures. Visceral organs are uninvolved even late in the course.

8. Prostate cancer can metastasize to the bone via Batson's plexus of paravertebral veins. There is also involvement of the lung, liver, endocrine glands and the central nervous sytem.

The main points from the cascade analysis have been summarized by Viadana *et al.* (1978a) as follows: (1) The overwhelming majority of cancers are disseminated by a multistep process. (2) Generalized disease (such as brain metastasis) does not ordinarily occur directly from the primary tumor. (3) There are one or more key generalizing sites that are specific for a given primary tumor. (4) These sites depend largely on the drainage of the venous blood. (5) The generalized disease is produced by secondary metastases from the key sites. (6) The similarity of the cascade processes at so many different sites reflects a similar underlying process of generalization through the blood system. To all such general points there will be occasional exceptions.

Given these data, one may ask why cancer cells metastasize step by step and do not generalize throughout the body directly from the primary tumor. It could be argued that cancer is a gradually developing disease. At the time

when the cells leave the primary tumor and enter the blood stream, they may not yet have evolved to the point where they can function effectively as the free-living cells that can grow almost anywhere in the body. Another explanation is given by Weiss and co-workers who recently investigated cancer cell traffic from the lungs to the liver (Weiss, 1980) and from the liver to the lungs (Weiss *et al.*, 1983). They propose that cancer cells which are temporarily arrested in the first organ encountered are "processed" by it, so that they die before or shortly after arrival in another organ. Mechanisms of tumor cell damage could include mechanical trauma, attack by NK cells, or damage due to cellular and humoral inflammatory responses to interactions of the cancer cells with vascular endothelium. It was suggested that metastases in second organs would, to a large extent, be generated by cancer cells from metastases in the "first organs" as distinct from direct seeding from cancer cells released from the primary tumor. These and other results to be discussed later (Section III,B,4) emphasize the potential importance of metastasis from metastases in the natural evolution and spread of cancer.

The underlying principle of the cascade hypothesis is that there are definite steps or stages in the evolution (Viadana *et al.*, 1978b; Bross, 1980). Although a number of questions remain unanswered, the cascade theory represents at least the beginning of a comprehensive scientific concept of metastasis, which can take into account stepwise changes in cancer cells, changes in the host environment and its defense system, as well as physical factors such as the anatomical and biochemical aspects of the routes of dissemination.

Apart from this, the findings of distinct metastatic pathways and of sequential development in human cancer raise a number of interesting questions that deserve intense future investigations:

1. Why are the lung and the liver such important key sites and not, for instance, the lymph nodes or other organs?
2. Are there changes occurring at the first metastatic site that make the tumor cells more adaptable for growth at other sites in the body?
3. Why are lung metastases so often the last step in the cascade process before the cancer generalizes to the endocrine glands, the central nervous system, and to other sites involved in the last stages of the disease?
4. What is the reason for organ predilection in metastasis? While for many years it was thought that metastases appeared in the organs in which they might be expected to occur on the basis of anatomy of the circulation, clinicians know of many exceptions. For instance, both Wilms' tumor and neuroblastoma drain into the inferior vena cava yet the predominant metastatic site for Wilms' tumor is the lung and for neuroblastoma it is bone. Other examples have been reported (Kinsey, 1960). Concepts for mecha-

nisms have been developed from experimental studies and will be discussed under Section III,B (Table IV).

B. Experimental Data

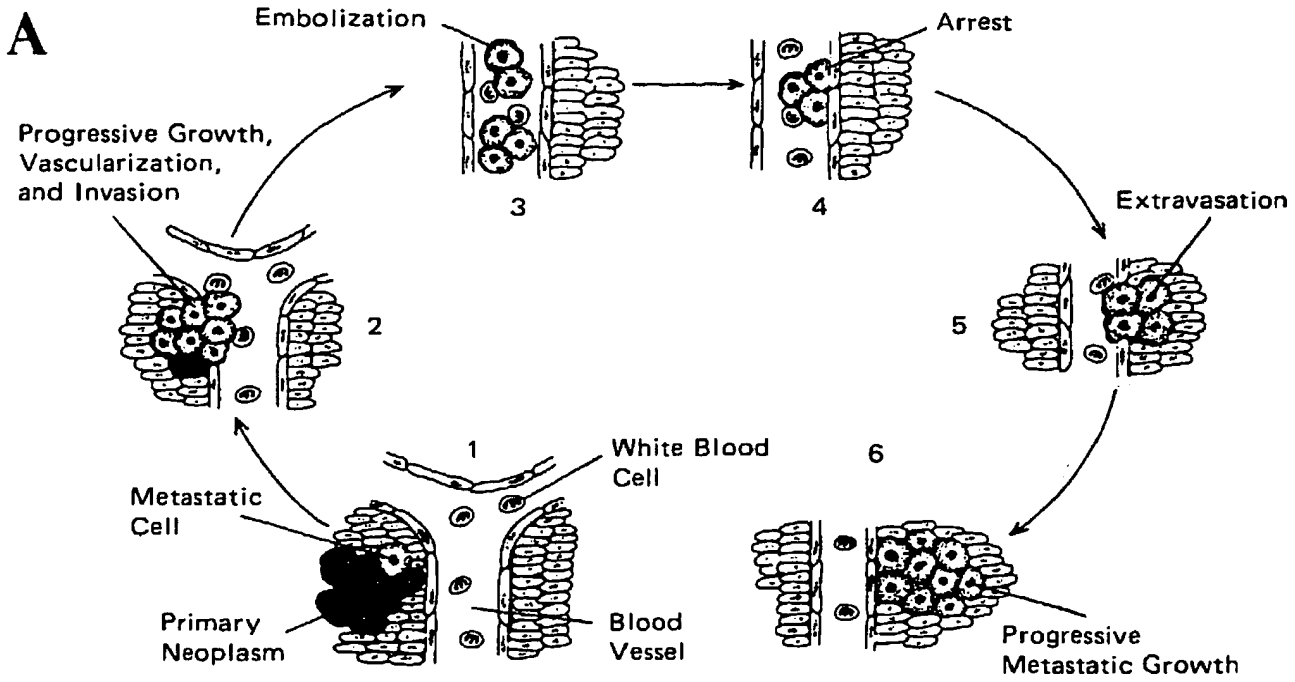
The cascade theory of metastasis has also formed the basis of a large number of experimental studies in the field. Here, analysis of functional aspects and of mechanisms are of primary concern. There is a surprisingly concordant view among experimentalists that the overall process of metastasis take place in sequential steps.

Subsequent to the establishment of a primary tumor, there must be invasion of the surrounding tissue with the eventual penetration of blood and/or lymphatic vessels. Circulating tumor cells or tumor emboli that eventually survive in the blood stream can stop in capillaries of distant organs. To establish secondary lesions, they must penetrate the vessel wall, infiltrate the surrounding parenchyma, and be able to grow there. The whole process may then be repeated starting from the metastatic lesion (Sugarbaker *et al.*, 1971; Hoover and Ketcham, 1975).

An analysis of the various steps that lead from a primary tumor to local or distant secondary lesions has been presented in several excellent reviews which should be consulted for details (Weiss, 1976; Nicolson, 1978a, 1979; Poste and Fidler, 1980). Figure 4 shows two illustrations of the process reproduced from Burger (1980) and from Fidler and Poste (1982).

1. Metastatic Inefficiency

It has been shown that only 1% or less of the millions of tumor cells that may escape from the primary tumor into the circulation survive to become a viable metastasis, while the majority of cells will die (Liotta *et al.*, 1974). It is thus an apparent paradox that on one hand metastasis is a major cause of death in patients with cancer, but on the other hand in terms of cancer cells themselves, metastasis appears to be an inefficient process (Weiss, 1980). Since the vast majority of cells released into the circulation will die, the primary tumor must reach a critical population size and/or growth rate to deliver sufficient cells into the circulation to proceed to the next step in the cascade. Most clinical metastases do not occur until the primary tumor has reached at least 1 cm^3, corresponding to about 10^9 cells (Weiss, 1982). As causes of the high rate of cell death of circulating nonlymphoid tumor cells, the following have been suggested: (1) Mechanical shear forces, (2) loss of attachment substrate and spreading, (3) oxygen toxicity, and (4) destruction by host-derived circulating natural killer cells. In animal experiments, the number of final metastases has been found to be proportional to the number of circulating tumor cells but the best correlation was with clumps of tumor



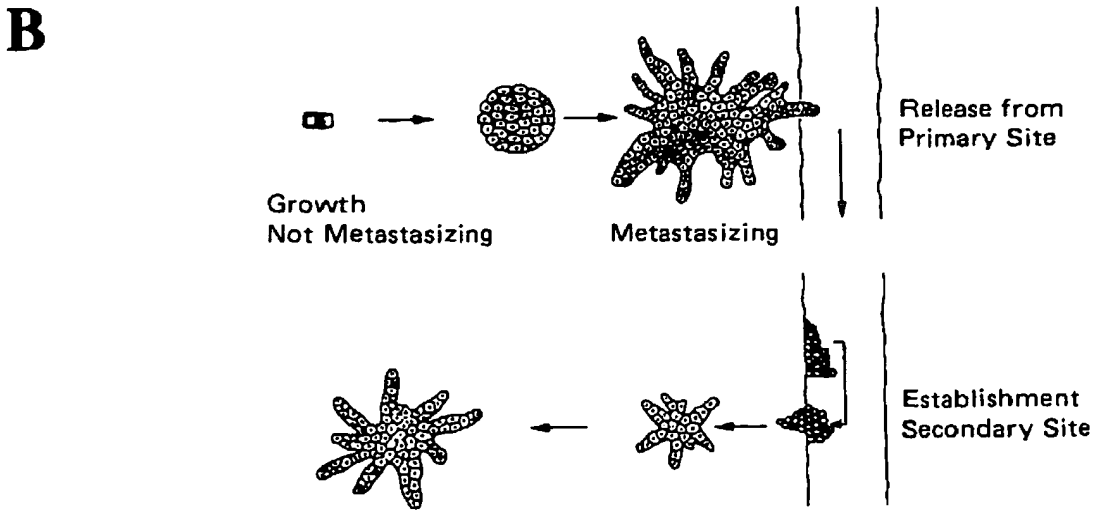


FIG. 4. Sequential ("cascade") steps in metastasis. (A) The process of hematogenous metastasis formation is dissected into six steps. (From Fidler and Poste, 1982.) (B) The process of metastasis is dissected basically into two topographically separate processes. The upper portion shows the process leading to penetration of a carrier system (blood, lymph, body cavities). After dissemination, the lower portion shows the processes that lead to the establishment of metastatic lesions in the periphery. (From Burger, 1980.)

cells. The experimental systems for studying the behavior of circulating tumor cells and their survival have been reviewed elsewhere (Fidler, 1976, 1978; Warren, 1981).

Shedding of tumor cells at an adequate rate into the circulation is not sufficient per se for metastasis. The primary tumor may have to pass through several stages of increasing malignancy before its cells are capable of metastasizing. Thus, in 1973, Fidler proposed that survival and eventual metastasis of B16 melanoma cells are dependent upon properties unique to the tumor cells and that the metastatic process is not random (Fidler, 1973). Similar observations were made in some tumor systems but not in others (Giavazzi *et al.*, 1980; Ryd *et al.*, 1983). The question of whether processes in metastasis are random or nonrandom is being discussed over and over again (Weiss, 1983). Most likely metastasis is neither an exclusively random nor an exclusively nonrandom process.

A systematic analysis of the colonization potential of spontaneous mouse mammary tumors (Tarin and Price, 1981) after inoculation of one million viable tumor cells ip, sc, iv tail vein, or iv hepatic portal vein revealed that each tumor line had its intrinsic colonization potential. The expression of this, however, was influenced by the microenvironment of an organ, for instance, its circulatory anatomy. The degree and sites of colonization were thus the results of interactions between tumor- and organ-specific factors.

2. *Organ Site Predilection*

Apparently contradictory hypotheses have been put forward to explain the selective involvement of certain organs in certain types of cancer: The "seed and soil" hypothesis and the "mechanistic theory." While Paget (1889) postulated that site-specific metastasis was the consequence of the provision of a fertile environment (the soil) in which compatible tumor cells (the seed) could proliferate, Ewing (1928) stated that site-specific metastasis was a direct consequence of the anatomical location of the primary tumor and of hemodynamic circumstances. Sugarbaker (1981) suggested that the mechanistic theory could explain phenomena at a relatively early stage of spread, whereas the seed and soil hypothesis might explain phenomena at later stages of disease progression. That tumor cell surface properties are important for organ selectivity was suggested by Hagmar (1972) and later by Fidler and Nicolson (1976). A theoretical analysis was performed by Weiss (1975), who suggested that the phenomenon of organ predilection is due to the ability of tumor cells to discriminate between various vascular beds during their travel in the circulation. Evidence for specific aggregation of organ-selected tumor cells with cell suspensions of the target organs—be they lung, liver, or ovary—was indeed obtained (Nicolson and Winkelhake, 1975). Similarly, using a cryostat section-binding assay, it could recently be shown that tumor lines would preferentially bind to sections from those

organs to which they also metastasized *in vivo* (Netland and Zettler, 1984; Kieran and Longenecker, 1984). The specific site of organ–tumor cell interaction remains to be elucidated, however. Recent findings point toward a role of tumor cell recognition via organ-specific receptors (Schirrmacher *et al.*, 1980; Cheingsong-Popov *et al.*, 1982) or lymph node homing receptors (Stevens *et al.*, 1982).

In a series of elegant organ ectopic site experiments, it could be shown that tumors that preferentially colonize the lung but not the kidney would do so also when tissue fragments of these two organs were implanted at an ectopic site (Hart and Fidler, 1980). Organ-determined modulation of tumor growth was furthermore indicated by experiments of I. R. Hart (1982) and M. M. Burger (personal communication). Such experiments seem to corroborate Paget's theory from nearly a century ago! The different concepts of mechanisms of site-specific metastases are summarized in Table IV.

3. *Extravasation*

Of importance for the process of extravasation seems to be the much higher affinity of circulating tumor cells to exposed matrix from the basal lamina as compared with the surface of blood vessel endothelium (Nicolson, 1978b; Vlodavsky *et al.*, 1982, 1983a,b). A confluent sheet of vascular endothelium in culture was shown to separate in response to contact with a fibrin clot (Kadish *et al.*, 1979) and to retract in response to tumor cells (Nicolson, 1978a). A similar retraction response to tumor cells was observed with mesothelium of the diaphragm (Haemmerli and Sträuli, 1978; Granzow *et al.*, 1980). Such a response may lead to exposure of a window of matrix to which tumor cells could stick and through which, after degradation, they could leave the circulation. Organ site predilection could be influenced by the composition of the subendothelial extracellular matrix in different organs.

TABLE IV
CONCEPTS OF MECHANISMS OF ORGAN SITE-SPECIFIC METASTASIS

Concepts	References
"Seed and soil" hypothesis	Paget (1889)
Mechanical entrapment hypothesis	Ewing (1928)
Combinations of 1 and 2	Sugarbaker (1981), Proctor (1976)
Specific adhesive interactions	Nicolson and Winkelhake (1975), Shearman *et al.* (1980), Schirrmacher *et al.* (1980), Cheingsong-Popov *et al.* (1983), Netland and Zetter (1984), Kieran and Longenecker (1983)
Organ-determined modulation of tumor growth	Tarin and Price (1981), Hart (1982)

4. *Metastases from Metastases*

A clinically important question is whether metastases can further metastasize because the decision about whether and when to remove metastatic foci depends partly on their threat as a source of new metastases. To answer this question, Hoover and Ketcham (1975) performed paraboisis experiments. They amputated primary tumors from mice after pulmonary metastases were present and then sutured two animals together along their side so that skin, muscle, and peritoneum were united. Within 5–7 weeks, pulmonary metastases developed in the test mouse, indicating their origin from the pulmonary metastases of the donor mouse.

5. *Interactions between Primary and Secondary Lesions*

There seem to exist negative feedback mechanisms in otherwise uncontrolled tumor growth. Sugarbaker *et al.* (1977) showed (1) that a primary tumor can inhibit the growth of metastases, (2) that the strength of inhibition is proportional to tumor mass, and (3) that inhibitory influences of an expanding tumor mass are probably also exerted upon itself and on a second tumor implant. Such inhibitory influences could be partially due to concomitant tumor immunity and partially to nonimmunological mechanisms, as has been reviewed recently (Gorelik, 1983).

6. *Important Properties in the Metastatic Cascade*

Factors of potential importance in the complex and dynamic process of metastasis are listed in Table V. They are subdivided according to tumor or host contribution and are by no means complete. Processes that promote the invasive capacity of a tumor and those that increase the resistance to natural and specific immune defenses will be advantageous for metastatic spread all along the metastatic cascade. A decrease in homotypic adhesion among the

TABLE V
SOME FACTORS OF POTENTIAL IMPORTANCE IN THE METASTATIC CASCADE

Factor	Contribution from	
	Tumor	Host
Growth rate and mechanical pressure	+	−
Tumor cell surface properties	+	−
Tumor cell adhesive properties	+	−
Migratory capability and deformability	+	−
Lytic enzymes	+	+
Fibrous reaction ("barrier effect"), invasion, and tissue resistance	+	+
Angiogenesis	+	+
Host immune responses and tumor immune escape	+	+

tumor cells will most probably promote release from the primary site. Conversely, an increase rather than a decrease in homotypic cell adhesion may promote tumor implantation and establishment in the periphery since cell aggregates trapped in a microvascular bed seem to escape the unsuitable environment of the blood stream much better than single cells. It thus seems that tumor adhesive properties have to change during different steps of the metastatic cascade. Host properties, in particular immunological reactions, could also have opposite effects resulting in either tumor inhibition or tumor enhancement. Release of lytic enzymes by inflammatory cells may be important for host defense reactions but it could also prepare host tissue for tumor invasion. Antibodies as well as T cells or macrophages have been shown to have tumor inhibitory effects in some situations and tumor-enhancing effects in others. The outcome may depend on subtle differences between subpopulations of the tumor cells as well as between subpopulations of the host's responding cells.

IV. Instability, Subpopulation Interactions, and Tumor Progression: Experimental Findings and Theoretical Concepts

One of the most insidious characteristics of cancer cells is their capacity to diversify and to create cellular variants. Even if a cancer originated from a single transformed cell as suggested by enzyme marker analysis (Fialkow, 1976), a tumor cell population at the time of clinical manifestation will be phenotypically and genotypically heterogeneous. Tumors may be heterogeneous in several ways: (1) heterogeneity among cancers of the same histological type coming from different individual patients, (2) heterogeneity arising over time by tumor progression within the same patient, i.e., from tumorigenic, noninvasive via invasive, nonmetastatic to invasive and metastatic subpopulations, (3) heterogeneity within a single tumor at any one time as revealed by histological examination; this includes differences due to microenvironmental conditions (stroma reaction, microvasculature, oxygen tension, pH, substrate supply, waste drainage) and due to stage of cell cycle.

Studies in several tumor systems have meanwhile confirmed Fidler's original observation (Fidler, 1973) that malignant tumors are heterogenous in metastatic capacity (Poste *et al.*, 1982). New observations have been made in the last few years concerning the stability of metastatic subpopulations or clones. When assessing the stability of the metastatic phenotype in clonal lines of B16 melanoma exhibiting high or low metastatic potential, it was found that the highly metastatic cells were often less stable (Fig. 5A). In the KV-2237 fibrosarcoma, a highly metastatic clone cultured for 60 days had become composed of subclones exhibiting a wide range of metastatic phenotypes, whereas a clone with low metastatic potential had retained this

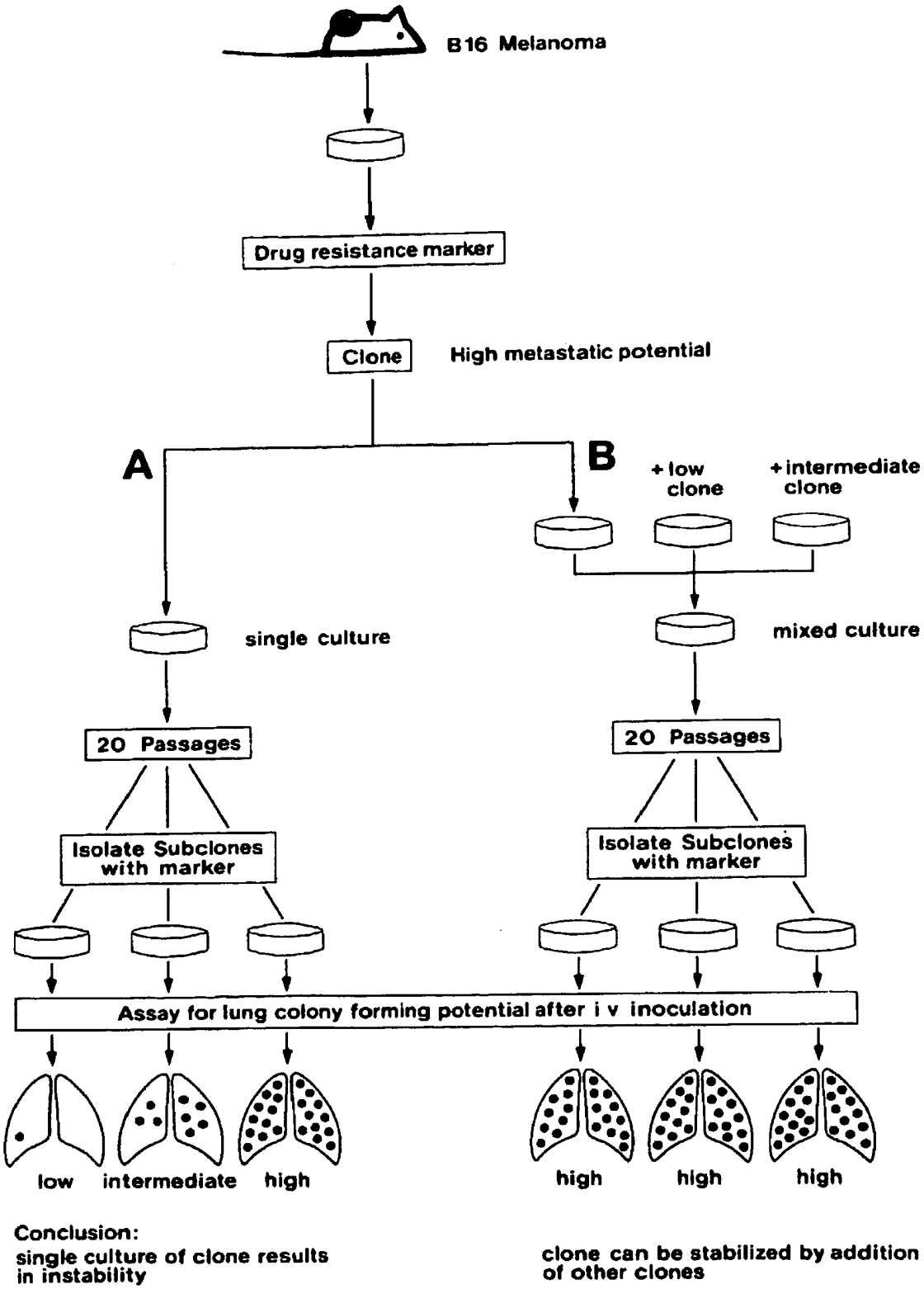


FIG. 5. Experimental evidence for clonal instability of the metastatic phenotype (lung colonization capacity) and of stabilization by subpopulation interactions. (Based on Poste *et al.*, 1981.)

phenotype (Poste *et al.*, 1981). This, however, must not always be the case. The experience of several groups (Chambers *et al.*, 1981; Neri and Nicolson, 1981; Miller *et al.*, 1983) shows that individual subpopulations and clones thereof are heterogenous in their stability. Furthermore, tumor subpopulation changes can be sudden or gradual, and they can result in either more or less malignant phenotypes (Heppner and Miller, 1983). Changes can occur after *in vitro* or *in vivo* passage. Clonal analysis of metastatic lesions produced by B16 melanoma populations containing clones with identifiable markers revealed that the majority of experimental metastases produced by intravenous injection of tumor cells were unicellular in origin (Talmadge *et al.*, 1982; Poste *et al.*, 1982). During the early stages of their growth (< 25 days), the majority of metastatic lesions contained cells with indistinguishable metastatic phenotypes (intralesional clonal homogeneity), while progressive growth (> 40 days) of metastatic lesions was accompanied by emergency of variant tumor cells with altered metastatic properties (intralesional clonal heterogeneity) (Poste *et al.*, 1982; Talmadge *et al.*, 1984).

There is, thus, no question that tumors are heterogeneous for invasion, metastasis, and various other biological properties. What is not understood are the mechanisms responsible for the generation of heterogeneity in primary neoplasms and among and within metastases. This is an important issue because it directly relates to tumor progression, i.e., heterogeneity seen in the same tumor as a function of time. How can we be successful in the long run in therapeutic intervention with metastasis if we do not understand mechanisms of progression? In the light of what we know so far about these processes, it seems quite possible that chemotherapeutic drugs, irradiation, and surgery, which all aim at reducing the overall tumor burden at the same time, affect the heterogeneity and change the biological behavior of the remaining tumor subpopulations.

Different concepts of mechanisms of tumor progression which are under present discussion are summarized in Table VI. The concept that malignant cells appear as the result of progressive stepwise changes was first introduced by Foulds (1975). It was then refined by Nowell (1976), who suggested that the process of tumor progression was the result of acquired genetic lability within tumor cells allowing for continuous selection of variant sublines, a neo-Darwinian concept which could explain several experimental findings (Chow *et al.*, 1983).

Genetic errors could arise from classical genetic mechanisms or from the production of cellular variants as in normal tissue differentiation. The type of genetic errors in neoplastic cells could include mutations in structural genes, mutations in regulatory genes, or major genomic changes due to numerical or structural chromosomal alterations. Nowell (1982) proposed a special role of oncogenes in this process, suggesting that variability in number and place of insertion sites could result in position effects on gene regulation.

TABLE VI
CONCEPTS OF MECHANISMS OF TUMOR PROGRESSION

Concepts	References
1. Progression due to stepwise neoplastic development through qualitatively different stages	Foulds (1975)
2. Progression due to acquired genetic lability allowing for continuous selection of variant sublines	Nowell (1976, 1982)
3. Progression dependent on controlling elements from within (clone-dependent genetic programming for shifts) and from without (regulatory influences via tumor subpopulation interaction and via inductive signals from the microenvironment)	Vaage (1980), Schirrmacher (1980), Neri and Nicolson (1981), Poste *et al.* (1981), Kiang *et al.* (1982), Katzav *et al.* (1983), Bennet (1983)
4. Progression due to changing environmental conditions; evidence for coexistence of hormone-dependent and -independent subpopulations prior to progression	Sinha *et al.* (1977), Sinha (1981), Sluyser *et al.* (1981), Isaacs and Coffey (1981), Isaacs *et al.* (1982)
5. Progression due to spontaneous somatic hybridization with host cells followed by chromosome segregation	De Baetselier *et al.* (1981), Dennis *et al.* (1981b), Kerbel *et al.* (1983)
6. Epigenetic mechanisms of tumor progression via changes in DNA methylation; induction of high-frequency heritable but phenotypically unstable changes	Frost *et al.* (1983), Kerbel *et al.* (1984), Feinberg and Vogelstein (1983)

Two recent findings seem to have particular relevance for the evolution of cellular diversity and metastatic heterogeneity within neoplastic lesions. (1) Highly metastatic cells were found to have higher mutation rates than low metastatic cells (Cifone and Fidler, 1981). (2) Heterogeneity was found to develop rapidly in cultures containing only one or a few subpopulations of cells. The addition of other subpopulations from the same tumor caused stabilization of the metastatic phenotype as tested in an experimental metastasis assay (Poste *et al.*, 1981). These findings as analyzed with the highly metastatic B16 melanoma are illustrated in Fig. 5B. Observations of clonal instability and clonal subpopulation interactions have been made in various tumor systems both *in vitro* and *in vivo* (Miller *et al.*, 1983; Poste, 1982; Poste and Nicolson, 1983).

The observations of clonal instability and of high mutation rates in highly metastatic cell lines support Nowell's hypothesis of progression being due to acquired genetic lability. The observations of subpopulation interactions introduce a controlling factor from the cells' microenvironment. Tumor cell subpopulation interactions can influence each other's growth behavior (Mil-

ler *et al.*, 1980), drug sensitivity (Miller *et al.*, 1981), immunological properties (Nowotny and Grohsman, 1973; Miller and Heppner, 1980), and metastatic phenotype (Poste *et al.*, 1982; Miller, 1983). They may be exerted via many mechanisms, including (1) metabolic cooperation, a process by which small molecules pass between cells in contact, presumably through gap junctions (Subak-Sharp *et al.*, 1969; Loewenstein, 1979), (2) growth factors and chalones secreted by tumor cells which could influence growth of other cells, and (3) host-mediated mechanisms of immunological nature (Chow and Greenberg, 1980; Miller and Heppner, 1980).

There have been other observations which suggest that genetic variability and selection may not be the whole story in tumor progression. For instance, Smith and Sanger (1982) described genomic rearrangements during tumor development that were nonrandom and were precisely reproducible from experiment to experiment. Table VII contains a list of observations where tumor cells were found to shift in their metastatic phenotype, often in a very characteristic manner, far from random. For instance, Vaage (1980) has shown in repeated serial transplantation experiments that individual C3H mammary tumors undergo "progression" in a highly reproducible way: Certain characteristics appeared in the same generations, as if on schedule. Similar observations of shifts or drifts were made by Neri and Nicolson (1981), Schirrmacher *et al.* (1982a–c, 1983), Dennis *et al.* (1981b), and Katzav *et al.* (1983). When studying stability of tumor antigen expression, we observed a clone-dependent variation of tumor antigen expression with time in tissue culture (Schirrmacher and Bosslet, 1982). During successive ip passage *in vivo* of uncloned as well as cloned low metastatic parental Eb-type cells, we repeatedly observed a phenotypic shift toward the high metastatic ESb variant phenotype (Schirrmacher *et al.*, 1982c, 1983). Such a shift was associated with changes in the expression of tumor antigens, differentiation antigens, and of Fcγ receptors. Table VIII contains data of such a shift in metastatic properties involving multiple phenotypic traits. Another interesting observation of shifts in metastatic capacity associated with a cell surface phenotype change was recently reported from the T10 ($H\text{-}2^k \times H\text{-}2^b$) F_1 sarcoma (De Baetselier *et al.*, 1980; Katzav *et al.*, 1983). Serial transfer of $H\text{-}2^k$ negative low metastatic clones eventually resulted in the appearance of high metastatic variants that concomitantly expressed $H\text{-}2^k$ antigens.

These observations of shifts in metastatic phenotype could be interpreted in such a way that there is a clone-dependent genetic programing for shifts on one hand and regulatory environmental signals on the other that influence progression. Progression may thus depend on controlling elements both from within the cell and from without (Table VI,3.)

Fould's original definition and rules of progression were based on extensive experimental and clinical documentation of how cancers behave. They

TABLE VII

PHENOTYPIC SHIFTS IN METASTATIC PROPERTIES AND POSSIBLE MECHANISMS

Observation	Tumor system	References
C3H mouse mammary carcinoma	Progression of individual tumors during serial transplantation highly reproducible. Clone dependent genetic program?	Vaage (1980)
GR mouse mammary tumors	Cyclical variability during progression toward hormone independence. Regulatory mechanisms among various subpopulations?	Kiang *et al.* (1982)
B16 melanoma	Genetic variability *in vitro* subject to regulatory influences involving tumor subpopulation interactions	Poste *et al.* (1981)
13762 adenocarcinoma and B16 melanoma	Clone-dependent phenotypic drift of metastatic and cell surface properties during growth in tissue culture	Neri and Nicolson (1981), Miner *et al.* (1982)
Eb/ESb lymphosarcoma	Repeated observations of shifts during serial transplantation of low metastatic Eb-type cells toward high metastatic ESb-type cells.	
	Shifts associated with the same changes in tumor antigen and differentiation antigen expression. Induction by signals from microenvironment?	Schirrmacher (1980), Schirrmacher *et al.* (1983)
MDW4 ($WGA^R \rightarrow WGA^S$)	High-frequency shifts from lectin-resistant nonmetastatic to reexpression of lectin-sensitive and metastatic phenotype. Due to fusion with host cells followed by chromosome segregation.	Dennis *et al.* (1981)
T10 ($H\text{-}2^b \times H\text{-}2^k$) sarcoma	Serial transfer of $H\text{-}2^k$-negative clones ends in appearance of $H\text{-}2^k$-positive cells concomitantly with acquisition of high metastatic capacity.	Katzav *et al.* (1983)

TABLE VIII

SHIFTS FROM Eb TO ESb DURING SUCCESSIVE ip PASSAGE OF A TWICE-CLONED Eb CELL LINE

Tumor line		Specific cytotoxicity with CTL (%)[a]				Metastatic[b] capacity	Surface markers[c]		
		Anti-Eb	Anti-ESb	Anti-H-2^d			Thy 1	Lyt 1	Fcγ R
Eb Cl 34.2 ⊙⊙⊙	PA1	35	5	79		0/20	+	−	−
	PA3	38	2	71	Progression ⊙ $TATA_{Eb}$				
	PA4	25	3	75					
	PA5	1	70	88	● $TATA_{ESb}$	34/40	−	+	+
	PA6	3	67	77					
Eb control		36	7	66		1/40	+	−	−
ESb control		4	77	80		35/40	−	+	+

[a] Percentage specific ^{51}Cr release in a 4-hr assay at a 40:1 effector:target cell ratio.

[b] Frequency of metastases in liver, lung, spleen, or kidney 12 days after sc inoculation of 10^5 tumor cells into syngeneic DBA/2 mice.

[c] Expression of lymphoid differentiation antigens (Thy 1, Lyt 1) analyzed by cytofluorograph; expression of Fcγ R (receptor for IgG) determined in an EA rosette assay.

were descriptive and did not suggest mechanisms. While later concepts of mechanisms (Table VI,2,3) suggest that progression occurs because new characteristics are acquired during tumor growth, there is still an extreme alternative possibility (Table VI,4), namely, that progression occurs because of changing environmental conditions. Such a situation may indeed exist in hormone-dependent cancers, where evidence was presented, even at the DNA level, for the coexistence of hormone-dependent and hormone-independent subpopulations prior to tumor progression (Michalides *et al.*, 1982). Isaacs and Coffey (1981) used fluctuation analysis to demonstrate the presence of androgen-independent cells within an androgen-dependent prostatic adenocarcinoma line. It may be interesting to note in this context that human prostatic cancer undergoes a morphological shift during transition to hormone independence (Sinha *et al.*, 1977).

High-frequency shifts from lectin-resistant nonmetastatic cells to reexpression of a lectin-sensitive and metastatic phenotype were reported by Dennis *et al.* (1981a,b), who studied the MDAY-D2 mouse tumor system. It could be shown later (Lagarde *et al.*, 1983; Kerbel *et al.*, 1983) that these shifts were due to a fusion of the tumor cells during local growth with host cells followed by chromosomal segregation. From the many possible segregants, those with metastatic properties were selected by the host and could be recovered from internal organs. The procedure for providing evidence for this type of mechanism is illustrated in Fig. 6. While several investigators have described fusion events between tumor and host cells *in vivo* (Goldenberg *et al.*, 1974; Lala *et al.*, 1980), it is only relatively recently that evidence was presented that such a process could lead to the generation of highly metastatic tumor variants (De Baetselier *et al.*, 1981, 1984; Lagarde *et al.*, 1983). Such a process could also be responsible for the interesting finding of Kerbel and associates (Kerbel *et al.*, 1980; Frost *et al.*, 1981) who reported that injection of strain A mouse tumors into DBA/2 mice resulted in the production of high metastatic DBA/2-type tumors. Progression could thus also be mediated by spontaneous somatic hybridization (Table VI,5) followed by selection of highly malignant segregants. One could imagine that a tumor–host cell hybridization event could lead to major genomic changes involving chromosomal rearrangements, unequal distribution of chromosomes, and chromosome losses during cell division (Larizza and Schirrmacher, 1984). We are intensively investigating such a mechanism in our laboratory as a possible basis for tumor progression in the Eb/ESb model system (Larizza *et al.*, 1984a,b).

In addition to genetic factors there seem to be microenvironmental factors involved in the generation of tumor heterogeneity. Based on observations of high-frequency variant generations *in vivo* (Dennis *et al.*, 1981b; Bosslet and Schirrmacher, 1982) and of microenvironmental influences (Schirrmacher *et al.*, 1982a), we have proposed a model in which signals from the tumor

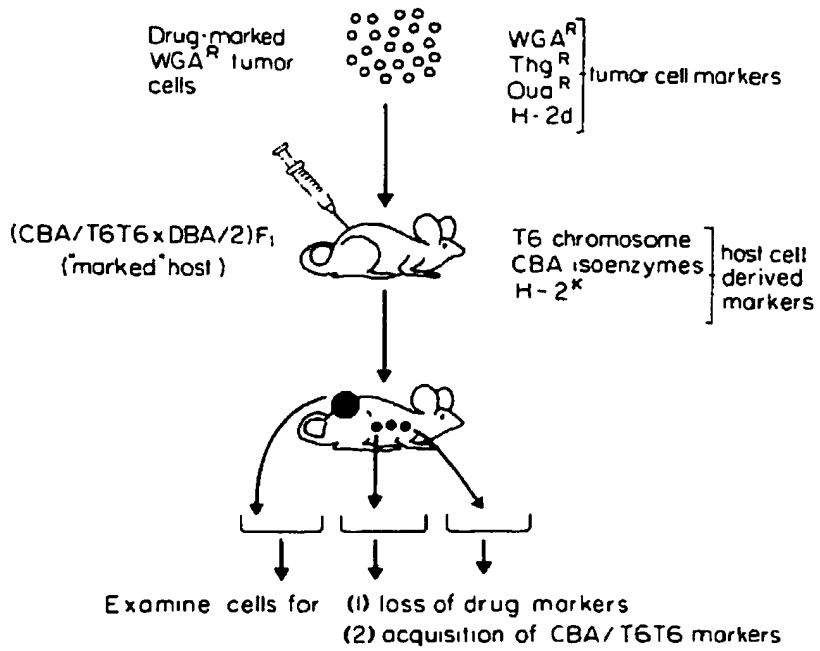


FIG. 6. Experimental approach that was used to test whether metastatic tumor variants were derived from a spontaneous fusion between tumor cells and host cells. (Reproduced from Kerbel *et al.*, 1982.) When drug-marked wheat germ-agglutinin-resistant low metastatic tumor cells were inoculated into genetically marked F_1 hybrid mice, metastatic wheat germ-agglutinin-sensitive variants developed which carried genetic markers from both the tumor and the host. (Lagarde *et al.*, 1983.)

microenvironment could activate genetic programs within tumor cell subpopulations causing phenotypic changes similar to those seen in normal tissue differentiation [Schirrmacher, 1980; see Fig. 7B (Altevogt *et al.*, 1982)]. In this model, the influence of the host is not only selective but also inductive. The model suggests (1) that multiple phenotypes can become expressed by one common genotype and (2) that microenvironmental soluble factors as well as cell–matrix contact (Reid, 1982) or cell–cell contact-mediated signals could have a regulatory role on tumor cell phenotypes and expression of heterogeneity. It should be kept in mind that heterogeneity is not a property exclusive to tumors but is seen in normal tissues as well. That neoplastic cells could give rise to variants through a process resembling normal tissue differentiation was first suggested by Pierce (1974) who found that single cells isolated from a teratocarcinoma differentiated *in vivo* into a wide variety of tissues representing all three germ layers. Bennet (1983) recently reported on differentiation and dedifferentiation processes in a cloned mouse B16 melanoma line which may be relevant to this discussion. Differentiation (pigment production) was followed in inducing media by time-lapse films allowing observations within single cells. Differentiation appeared unrelated to cell division and could be reversed in a proportion of cells. Dedifferentiation was associated with cell proliferation, so that most

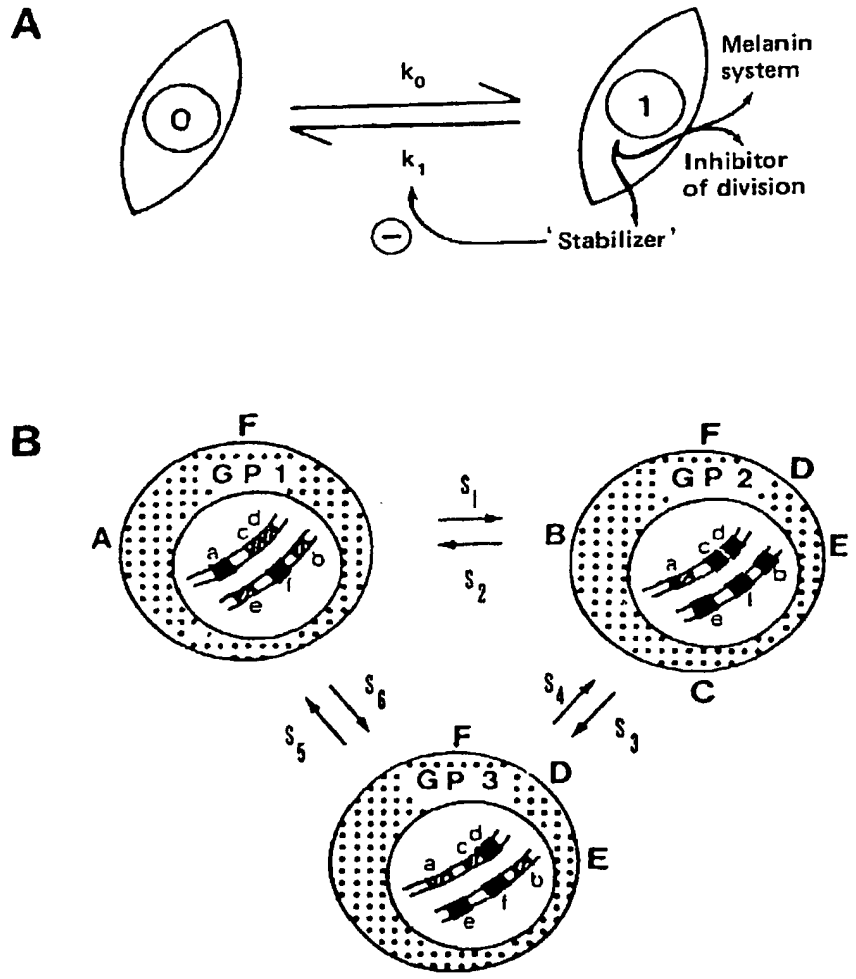


FIG. 7. (A) Model for the differentiation of melanoma cells; 0 and 1 represent states in which a set of functions associated with melanin synthesis are turned off and on, respectively. Cells can change their state stochastically either way with rate constants k_0 and k_1 (cells per cell per unit time). These rates will depend on the extracellular and intracellular milieux. (Reproduced from Bennett, 1983.) (B) Model showing how microenvironmental signals could cause shifts in tumor cell phenotypes by activating preformed genetic programs (GP1–GP3). A–F, Phenotypic markers; S_1–S_6, signals from the microenvironment. (Reproduced from Schirrmacher, 1980.)

pigmented clones were small and most unpigmented clones were large. These findings were accomodated by a model (Fig. 7A) in which functions associated with differentiation could switch on and off. It was further suggested that an inhibition of the "off" transition would build up in the "on" state. Also relevant in this context may be the findings by Raz and Ben-Ze'ev (1983) of modulation of the metastatic capability in B16 melanoma by cell shape.

During normal cell differentiation many genes are apparently switched off by DNA methylation. Tumor cells on the other hand seem to reexpress some of these genes, as exemplified by expression of embryonic antigens or by

production of "wrong" hormones. Gene activation by DNA demethylation could thus be yet another important factor in tumor progression (Table VI,6). This may be particularly relevant in the context of chemotherapy, where drugs are used, many of which are known to affect DNA methylation. The potential danger of facilitation of tumor progression by cancer therapy has been pointed out by Kerbel and Davies (1982).

V. Angiogenesis

Several important aspects of metastasis have not been discussed yet: angiogenesis, invasion, dormancy, and host immune responses. Because of their importance, a separate paragraph is devoted to each of these phenomena.

Most solid tumors appear to pass through two phases of growth. In the avascular phase, a tiny tumor nodule will grow up to a few millimeters in diameter only and usually will not be invasive. In the vascular phase, new capillary sprouts are induced from the host which grow toward the tumor. When these vessels penetrate the tumor, it begins to grow rapidly and to invade (Folkman, 1974a,b) (Fig. 8). The extensive vascularity of solid tumors has been recognized for over 100 years but little progress was made because of the lack of an experimental system with which to study angiogenesis. Only in the last 15 years has the importance of this phenomenon for autonomous tumor growth been demonstrated and only within the last 6 years has the possibility of chemical interference been apparent (Langer and Murray, 1982). This progress was made with the introduction of new model systems, such as (1) tumor implantation into the anterior chamber of the rabbit eye (Gimbrone *et al.*, 1974) (where tumors remain small because new vessels cannot reach them through the aqueous humor), (2) tumor incubation inside a millipore chamber (demonstrating that vascularization is induced by a diffusible chemical substance) (Greenblatt and Shubik, 1968), and (3) cartilage implantation on the highly vascular chick chorioallantoic membrane (demonstrating antiangiogenetic factors in a tissue into which tumors cannot metastasize) (Eisenstein *et al.*, 1975).

Among human neoplasms *in situ* carcinoma is most analogous to the avascular phase. *In situ* carcinoma of the cervix or bladder may exist for years and this prolonged period is commonly free of metastases. Almost simultaneously with neovascularization, *in situ* carcinomas start to invade through the tough basement membrane, (BM) (Folkman, 1976). There is also experimental support for a close relationship between angiogenesis by the host and invasion by the tumor so that it seems difficult to decide which of the two processes comes first. As shown in Fig. 8, there are wide gaps between the endothelial cells in the advancing tips of proliferating capillaries and the

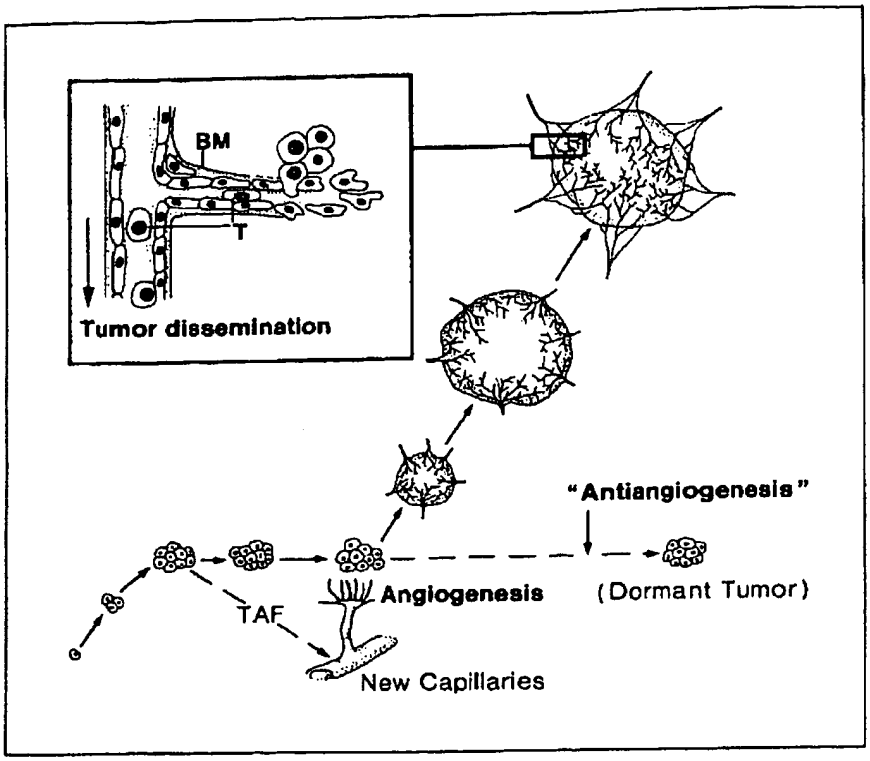


FIG. 8. Illustration of three concepts basic to tumor growth and metastasis. (1) Solid tumors pass through an avascular and a vascular stage. (2) New host capillaries are stimulated by a humoral angiogenetic factor (TAF) released by the tumor; rapid growth follows; upper left magnification shows endothelial cells at the tip of a new tumor-induced capillary, which originated from a venule; at the tip of the capillary sprout the basement membrane (BM) is fragmented so that tumor cells (T) from the primary mass may escape through these gaps into the circulation. (3) If angiogenesis is inhibited, further tumor growth is blocked and the tumor may enter a dormant phase. (Modified according to Folkman, 1974a.)

foremost part of the capillary has no basement membrane. This may explain the ease with which tumor cells can enter the circulation from the vascularized tumor (Fig. 8, inset).

The processes of angiogenesis and invasion have been analyzed in detail in the rabbit cornea model (Folkman, 1976). The original tumor implant grows slowly during the avascular phase. When new capillaries grow into the cornea, they are able to intrude between tightly packed layers of collagen, an invasive property that was not seen with the tumor cells themselves. Once the tumor has become vascularized, it is very destructive and capable of invading through all layers of collagen to the outer aspect of the cornea.

Folkman and associates have been able recently to culture human and bovine capillary endothelial cells, to clone them, and to study their growth and differentiation in long-term culture (Folkman and Haudenschild, 1980). The most interesting findings can be summarized as follows: (1) While aortic

endothelial cells can be grown in regular culture medium, capillary endothelial cells grow only slowly and then die. In tumor-conditioned medium, however, these cells grow rapidly with a doubling time of 28 hr and continue to proliferate for as long as the tumor-conditioned medium is present. (2) Cloned capillary endothelial cells, cultured in tumor-conditioned medium, form capillary tubes similar to capillaries *in vivo*, although there may be an inside-out type of conversion. The information necessary to develop an entire network *in vitro* with branch points and anastomoses thus seems to be contained within one cell type. It was recently shown that this whole process of capillary network formation is prompted also by collagen matrices (Montesano *et al.*, 1983).

Capillary endothelial cells will become very useful to distinguish between direct and indirect angiogenesis factors. Compounds like formic acid or silica which attract macrophages or other white cells might be recognized as indirect factors that are unable to directly induce capillary formation *in vitro* but which could stimulate other cells to do so. This *in vitro* procedure also will be likely to allow better characterization of antiangiogenesis factors and their mechanism(s) of action.

With regard to angiogenesis inhibition and its effect on growth of primary tumors and their metastases, exciting results have been recently obtained and reported by Folkman and his associates (Folkman *et al.*, 1983). They had previously demonstrated that angiogenesis *in vivo* can be promoted by heparin and inhibited by protamine, an antagonist of heparin (Taylor and Folkman, 1982). Now they report that oral administration of heparin resulted in the release of a nonanticoagulant heparin fragment in the serum, a hexasaccharide, which when given together with cortisone brings about a very potent inhibition of angiogenesis. Neither the heparin fragment nor cortisone alone showed this effect. When tumor-bearing animals were given heparin and cortisone (dissolved in their drinking water), it was found that large tumor masses regressed and metastases were prevented. Although evidence was given that the antitumor effect was due to angiogenesis inhibition, the mechanism of the synergistic effects of heparin and cortisone was not resolved. Also unexplained was the finding that many but not all tumors tested responded to this kind of therapy. It was suggested that the nonresponder tumor types may perhaps be able to degrade heparin or in some way interfere with the effect of heparin and cortisone on endothelium. Another substance that can block tumor angiogenesis *in vivo*, thereby restricting tumor growth, was recently reported to be present in shark cartilage (Lee and Langer, 1983).

Advances in the study of capillary endothelial cell growth *in vitro* and of powerful angiogenesis inhibition *in vivo* have thus demonstrated how re-

search focused on essential steps of the metastatic cascade can lead to a better understanding of mechanisms of metastasis formation and to new treatment strategies.

VI. Cancer Invasion

A. General Considerations

Higher organisms are composed of a number of tissue compartments that are separated from each other by extracellular matrices, such as basement membranes and interstitial stroma. During metastasis, tumor cells must traverse these matrix barriers as they cross tissue bounderies.

The basic structures that serve as barriers to invasion can be divided into three broad categories: (1) organ parenchymal cells, which include epithelium, endothelium, and mesothelium; (2) basement membranes, which separate these cells from the underlying stroma; and (3) connective tissue consisting of connective tissue cells embedded in their extracellular matrix proteins (see Table IX). Carcinoma is by far the most commonly occurring form of cancer and is a neoplasm of epithelial cell origin. Direct invasion is the first and most crucial step in the malignant process and is defined in carcinomata by local disruption of basal lamina with tumor cell infiltration into the underlying interstitial stroma. After traversing the stroma, tumor cells gain access to lymphatics and blood vessels for further dissemination. Tumor cells also have to cross basement membranes when they move into or out of blood vessels (intravasation or extravasation) such as venules and capillaries. In the target organ, where metastases are initiated, tumor cells that have extravasated must migrate through the perivascular interstitial stroma before colony growth occurs in the organ parenchyma. It is obvious that the extracellular matrix (ECM) is a mechanical barrier of the host that has to be penetrated at multiple stages during the metastatic process. The mechanism of tumor invasion is therefore studied mostly on the level of the interaction of tumor cells with ECM.

The basement membrane (BM)—probably the most important barrier—is a tough, elastic structure that is impermeable to colloidal carbon (0.5 μm). The insoluble nature of the BM is in part due to the unique arrangement of type IV collagen molecules (Miller, 1976; Timpl *et al.*, 1978) which are interconnected at their end regions to form a hexagonal network. In addition to collagen fibers, the BM matrix contains specific glycoproteins, such as fibronectin or laminin; proteoglycans, such as heparin, heparan sulfate, or chondroitin sulfate; and elastin (Kefalides, 1978). The ECM is thus a network consisting of a mixture of soluble and insoluble cross-linked mac-

TABLE IX

BIOCHEMICAL PARAMETERS OF HOST TISSUE PENETRATION BY METASTASIZING TUMOR CELLS[a]

Host tissue	Structural element	Biochemical components	Degradative enzymes
Epithelium Endothelium Mesothelium	Desmosomes Tight junctions Anchoring fibrils	Disulfide-bonded proteins	Plasmin Plasminogen activators Serine proteases
Interface between different tissues	Basement membranes	Fibronectin laminin Type IV and V collagens Proteoglycans	Mast cell serine protease Type IV collagenase, Type V collagenase } (metalloproteases) Heparanase (endoglycosidase)
Connective tissue stroma	Anchoring fibers Collagen fibers Elastic fibers	Fibronectin, glycoproteins Type I, III, and V collagens Elastin Proteoglycans	Cathepsins and thiol proteases Leukocyte elastase Classical collagenase
Bone Cartilage	Ground substance Mineralized matrix	Osteonectin, elastin Type I and II collagens Proteoglycans and GAGs Hydroxyapatite	

[a] For reviews see Liotta *et al.* (1982a,b), Jones and De Clerck (1982), and Pauli *et al.* (1983).

romolecules produced by specialized cell types. Detailed structural information of ECMs is an important prerequisite for understanding their function under normal and pathological conditions.

The basal lamina has been shown to be a product of epithelial cells that serves to stabilize epithelial cell differentiation and orientation during organogenesis. In addition, this structure is most likely important for cell anchorage *in vivo* and so may play a central role in growth regulation. As the early stages of oncogenesis involve deregulation of cell differentiation, orientation, and proliferation, it is possible that the gradual loss of basal lamina integrity that precedes its complete disruption during carcinoma development may be involved in neoplastic disorganization prior to the onset of malignant invasion. It is now known that the tumor can modify the matrix in the following ways: (1) tumor cell synthesis of matrix components, (2) degradation of matrix components associated with tumor invasion, and (3) stimulation of host cells to increased production of matrix components such as in desmoplasia (see Liotta, 1982). The matrix becomes locally permeable to cell movement not only during malignant invasion but also under normal physiological conditions, such as wound healing, tissue remodeling, neovascularization, and inflammation.

Information on tumor cell invasive behavior *in vivo* is mostly obtained from histologic and ultrastructural studies of fixed tissue sections because the tissues are normally opaque and thus exclude direct observations of tumor cells. There are a few exceptions, however, where the dynamics of tumor cell behavior have been followed *in situ* using transparent tissues such as mesentery, chorioallantoic membrane (CAM), rabbit ear chamber, hamster cheek pouch, and others (Amstrong, 1980). There are certain aspects of tumor invasion that can only be studied *in vivo,* in particular those that deal with the interference of host inflammation and other defense reactions. Such inflammatory reactions at the site of tumor invasion could favor the process by the release of tissue lytic enzymes and mitogenic or angiogenic factors from lymphocytes or macrophages. On the other hand, invasion could be retarded by the recruitment of inflammatory cells with tumoricidal or tumoristatic activity exerted by activated macrophages, NK cells, or T cells.

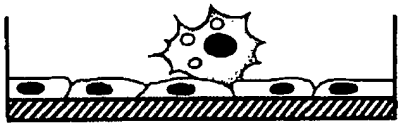
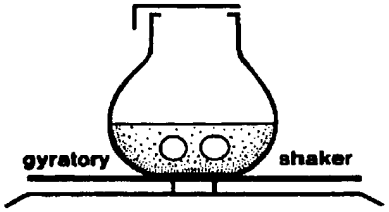
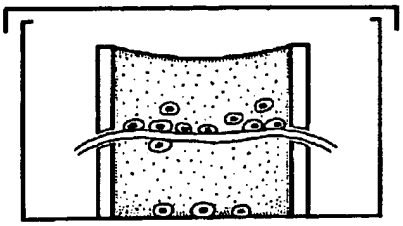
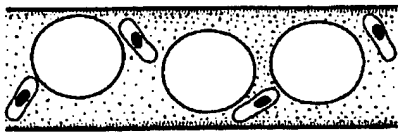
The correlation of the course of invasion with the type of host response is an important but very complex issue that obviously requires further improvement of technologies.

Different *in vitro* assay systems for studies on cancer invasion are listed in Table X. Their respective advantages or disadvantages have been discussed elsewhere (Liotta and Hart, 1982). All of the methods in current use are simplified versions of *in vivo* processes and undoubtedly far away from the natural situation. However, most of these studies are performed to overcome some of the major obstacles of the *in vivo* work. A main point concerns the

ability to quantify the kinetics and the extent of tumor invasion in an accurate and reproducible manner. For this particular purpose tumor cells have been labeled radioactively, for instance with ^{51}Cr, ^{99m}Tc, [^{3}H]thymidine, [^{125}I]iododeoxyuridine, and ^{35}Se-labeled methionine.

Another important advantage of some *in vitro* systems is the ability to recover the invasive tumor cells, for instance, after they have penetrated a basement membrane, and to compare them with noninvasive tumor cells from the same starting population. In view of the phenomena of clonal instability, tumor heterogeneity, and subpopulation interactions, it may become an important issue to quantify changes in the proportion of invasive cells at different stages of tumor development.

TABLE X
In Vitro Assays for Cancer Invasion

Tumor cells cocultured with	References
Endothelial cell monolayers, hepatocyte cultures, subendothelial ECM, extracted bone cartilage 	Liotta *et al.* (1977), Kramer and Nicolson (1979), Zamora *et al.* (1980), Jones and De Clerck (1980), Pauli *et al.* (1981), Roos *et al.* (1981), Vlodavsky *et al.* (1983a,b)
Organ cultures, tissue fragment cultures, rotation-mediated cell aggregates 	Easty and Easty (1974), Schleich *et al.* (1974), Scher *et al.* (1976), Pourreau-Schneider *et al.* (1977), Mareel *et al.* (1975, 1979), Noguchi *et al.* (1978), Schirrmacher *et al.* (1979), Lohmann-Matthes *et al.* (1980), Poste *et al.* (1980), Schirrmacher *et al.* (1982d)
Membrane penetration systems, two-chamber systems 	Hart *et al.* (1978), Tchao *et al.* (1980), Thorgeirsson *et al.* (1982)
Artificial vessel walls 	Jones *et al.* (1981), Bogenmann *et al.* (1983)

With regard to the type of normal tissue substrate used, different types of *in vitro* assays have been established (Table X). These are, for instance, monolayer cell culture systems, three-dimensional cell aggregates mediated by rotation, organ cultures of a variety of host tissues, basement membrane-containing systems, and artificially constructed tissues grown on filters or nylon meshes. The utility of any particular experimental model obviously depends on the question being asked.

An important point for the strategy of experimental analysis of tumor invasion is the requirement of parallel *in vitro* and *in vivo* studies. The tumor cells to be used *in vitro* have to be shown to be invasive *in vivo*. It has to be verified that the many changes that cells might undergo when initiated in culture do not affect the trait of interest, namely, their invasiveness *in situ*.

B. Sequential Steps in Cancer Invasion

A three-step hypothesis has been proposed (Liotta *et al.*, 1982a) to describe the sequence of biochemical events during tumor invasion of ECM (Fig. 9). The steps are (1) attachment to the matrix (probably involving tumor

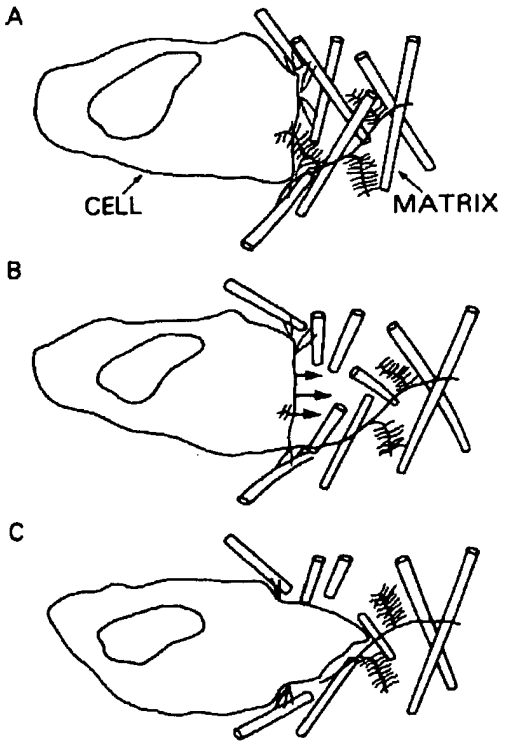


Fig. 9. Three-step hypothesis of tumor cell invasion of extracellular matrix. (A) Attachment may be mediated by specific attachment factors such as laminin (in the case of basement membrane) and fibronectin. (B) Local degradation of matrix by tumor-associated proteases. (C) Tumor cell locomotion into the region of matrix modified by proteolysis. (Reproduced from Liotta *et al.*, 1982a.)

cell surface receptors binding to specific adhesive glycoproteins), (2) local proteolysis, and (3) tumor cell locomotion into the region of the matrix modified by proteolysis (for reviews see Liotta and Hart, 1982; Pauli *et al.*, 1983; Jones and DeClerck, 1982; Sträuli *et al.*, 1980; Liotta and Hart, 1982).

Three major theories have been proposed for describing the pathological interactions between transformed cells and host stroma during tumor invasion: (1) the mechanical pressure theory, (2) the enzymatic theory, and (3) the migratory theory.

These concepts are not mutually exclusive, but may be interdependent and coordinated with one another. The relative contribution of each of these factors may vary depending on the type of tumor and the type of host stroma.

The mechanical pressure theory proposes that the increased pressure of expanding primary tumors causes rapidly proliferating tumor cells to force their way into alternative locations within host tissue. These possibly seek for ways of least tissue resistance and often migrate along natural cleavage planes such as collagen fibers, blood or lymph vessels, or nerves. Large-scale studies on autopsy material, however, show no evidence of a correlation between tumor size, mechanical pressure, and invasion and metastasis: There are examples of early metastases from very small primary tumors which could only generate small expansion forces, as well as examples of large tumors with pressure atrophy of the parenchyma and lack of metastases. Pressure thus probably plays only a minor role in tumor invasion in most cases.

The enzymatic and migratory theories gain support from a number of clinical and experimental observations. It appears reasonable to suppose that the translocation of a tumor cell from place A to place B in host tissues involves active locomotion. However, this can only be achieved when host barriers provided by extracellular matrices are altered to allow passage for the invading cells. Such alterations require the action of proteolytic enzymes. Table IX contains a list of the most common degradative enzymes involved in host tissue penetration by metastasizing tumor cells. Enzymatic action might be sufficient to focally lower the level of molecular organization and to reduce the physical resistance of host matrices. It has been shown for instance that proteinases that only act as collagen "cross-linkases" or "proteoglycanases" can transform the ECM from an insoluble into a more fluid state. A detailed model of matrix degradation has been proposed by Pauli *et al.* (1983). This is shown in Fig. 10.

Proteolysis of the host ECM can be achieved either by enzymes derived from the tumor cells themselves or by enzymes from host cells, such as vascular endothelial cells (in angiogenesis), fibroblasts, macrophages, and mast cells. It has been suggested that some tumors may elaborate factors that directly stimulate fibroblasts to increased collagenase synthesis and secretion (Bauer *et al.*, 1977).

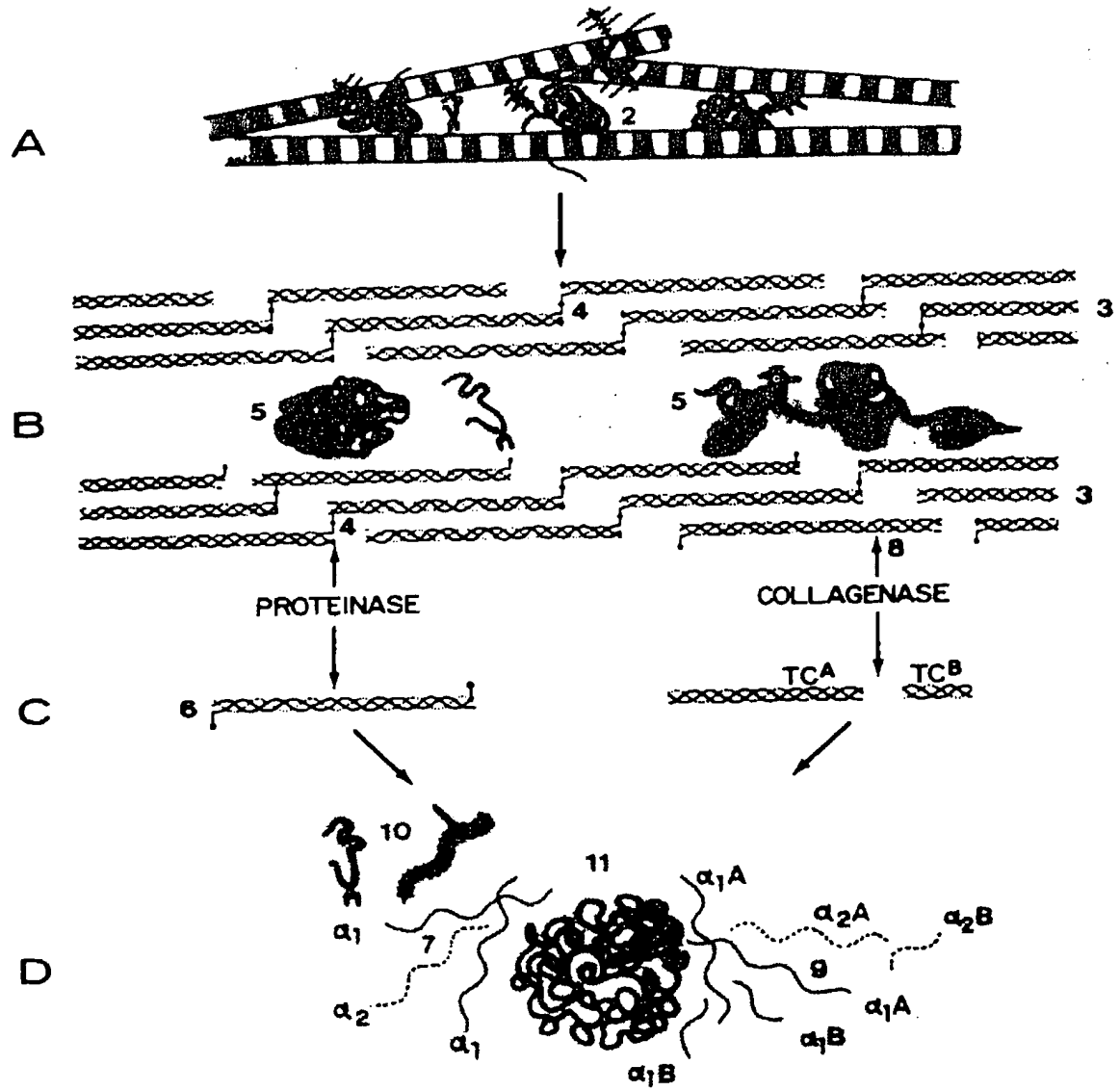


FIG. 10. Model of degradation of extracellular matrix (ECM) at the tumor invasion zone. (A) ECM consisting of collagen fibers (1) and GAGs (2) that provide swelling pressure. (B) Higher magnification of (A) showing covalent cross-links (4) between neighboring collagen molecules (3). Proteoglycans aggregated with hyaluronate (5) are restricted from swelling by the intact collagen network. (C) Collagen fibers are degraded by two enzymatic pathways: (i) proteinases (i.e., cathepsins, elastase, plasmin, thrombin) act as cross-linkages (4) to liberate collagen monomers (6) which then denature (7), solubilize and become susceptible to many proteinases; (ii) collagenases specifically cleave the collagen triple helix (8); the resulting fragments denature (9) and become further degraded. (D) Collagen and proteoglycan degradation (10) transforms the matrix from a solid to a fluid state thus allowing locomotion and tumor cell penetration. (Reproduced from Pauli *et al.*, 1983.)

The most popular proteolytic enzymes studied in relation to tumor invasion are collagenases (Liotta *et al.*, 1982a,b), plasminogen activators, cathepsins, elastases (Jones and DeClerck, 1980; Pauli *et al.*, 1983), and proteoglycan-degrading enzymes (Kramer *et al.*, 1982; Nakajima *et al.*, 1983;

Vlodavsky *et al.*, 1983b). A summary of biochemical parameters of host tissue penetration and the involvement of respective degradative enzymes is given in Table IX.

The migratory theory stresses the importance of cellular locomotion of tumor cells. This may be brought about by a creeping motion of isolated cells or by the moving boundary of a sheet of cells. Amoeboid deformability of single cells may allow them for instance, to insert an arm of cytoplasm (pseudopod) through endothelial cells as seen *in vitro* (Vlodavsky *et al.*, 1983c) and to transfuse the rest of the cell body through this hole. Migration may be initiated by loss of intercellular adhesion, facilitating separation of tumor cells from each other and allowing tumor cells with increased motility to invade normal tissues. Common experiences with cell separation procedures show that enzymatic dissociation of intercellular bonds provided by fibronectin, heparan sulfate, hyaluronate, or divalent ions may contribute to detachment of individual cells or cell clusters from solid tumors. Of special importance may be cell surface-associated proteinases which lead to increased cell dissociation, increased doubling times, and abnormal intercellular junctions in carcinoma cells *in vitro* (Pauli and Weinstein, 1982). Cell locomotion requires sequential attachments and detachments of localized areas of the cell surface to a substrate (Toole, 1981). Based on a biochemical analysis of the attachment sites (footpads) of cells, Rollins and Culp (1979) have postulated that cell substrate adhesion is mediated by fibronectin and heparan sulfate, whereas subsequent detachment, which is necessary for cell movement, involves hyaluronate and chondroitin sulfate. A role for hyaluronate in cell movement within a cellular stroma has been suggested from various studies of normal and tumor cells. It seems that tissue swelling ahead of migrating cell populations depends on the presence of large concentrations of hydrated hyaluronate. The studies of Toole *et al.* (1979) in the V2 rabbit carcinoma model have shown that the hyaluronate concentrations are most elevated in the invasion zone.

Tumor cell locomotion has been shown to be influenced by chemotactic factors (Romnaldez and Ward, 1975). Chemotactic factors can induce directional motility of tumor cells in the Boyden chamber assay. In addition, such factors can stimulate cell swelling and also foreign surface adhesiveness (Varani *et al.*, 1981; Lam *et al.*, 1981). In the Walker 256 carcinosarcoma, treatment with either the C5a-derived chemotactic peptide, the synthetic tripeptide NMFP (*N*-formylmethionylleucylphenylalanine), or with 12-*O*-tetradecanoyl phorbol ester induces a rapid, transient adherence response. From drug inhibition studies it was suggested that this adherence response is mediated by lipoxygenase metabolites of arachidonic acid (Varani, 1982).

In vivo experiments in animals bearing circulating tumor cells demonstrated that chemotactic factor injection caused a localization of tumor cells

at the site of factor application (Ozaki *et al.*, 1971; Orr *et al.*, 1981). It has therefore been suggested that the chemotactic response of tumor cells could play an important role in cancer metastasis (Lam *et al.*, 1981). Tumor cells localizing at metastatic sites *in vivo* may be influenced in a similar way as leukocytes, which become localized at sites of inflammation.

The sequence of events which characterize invasion can be summarized as follows:

1. *Tumor cell shedding.* Detachment of tumor cells from the primary mass could be brought about by loss of intercellular junctions (desmosomes), alterations in chemical composition and physical properties of the cell surface coat, and loosening of cell substrate interactions.

2. *Tumor cell attachment* to the matrix of interstitial connective tissue stroma or of basement membranes. Attachment may be mediated by specific adhesive glycoproteins such as collagens, laminin, fibronectin, and others. Laminin is a cross-shaped molecule (Timpl *et al.*, 1978) that has a binding site for a cell surface laminin receptor (Lesot *et al.*, 1983; Terranova *et al.*, 1983; Malinoff and Wicha, 1983) as well as binding sites for fibronectin and heparan sulfate. Fibronectin is an S–S-bridged two-chain protein molecule with domains for binding of collagens, heparin, actin, fibrin, and for cells (see Vartio *et al.*, 1983). Its possible role in cell adhesion and invasion has recently been discussed (Ruoslahti, 1984).

3. *Local proteolysis.* Increased proteolytic activities at the invasion front cause local alterations in the surrounding ECM making it easier to penetrate. Such proteases may be tumor cell or host cell derived and may degrade attachment proteins as well as the structural collagenous proteins of the matrix. Collagenases and cathepsins, elastase, and other neutral proteinases are the types of enzymes most frequently associated with matrix destruction and invasion. In some tissues this process appears to be regulated by natural inhibitors of proteases.

4. *Tumor cell locomotion.* Tumor cells migrate into the zones affected by proteolysis. They possibly move as aggregates along guidance tracks provided by host structures. Migration seems to be preceded by swelling of glycosaminoglycans, particularly hyaluronate, in the matrix of connective tissue (Pauli *et al.*, 1983).

C. Tissue Resistance to Invasion and Host Defense Reactions

While most loose connective tissues and bone are readily invaded by malignant tumors, cartilage and other avascular tissues such as aorta, heart valves, cornea lens, and epithelia are relatively resistant (Eisenstein *et al.*, 1973, 1975; Kuettner *et al.*, 1978; Waxler *et al.*, 1982). This selective resistance of certain tissues to invasion may be determined by structural properties as well as by tissue-specific antiinvasive factors (AIFs) (Kuettner *et al.*,

1977). Bovine nasal cartilage has been reported to contain at least three distinct proteinase inhibitors, an inhibitor of collagenases (MW 22,000), an inhibitor of thiol proteinases (MW 13,000), and an inhibitor of trypsin (MW 7000) (Rifkin and Crowe, 1975). Cartilage-derived AIFs also express inhibitory activities against neutral metalloproteinases that cleave collagen types IV and V and against human neutrophil elastase. In addition, AIF contains antiproliferative activity directed against vascular endothelial cells in culture and can inhibit tumor cell penetration of native connective tissue such as human amnion (Thorgeirsson *et al.*, 1982).

Another example of a host–tumor interaction is the deposition of fibrin which has been demonstrated within tumors by immunofluorescence and electron microscopy. It appears that tumors bring about fibrin deposition and remodeling in their own vicinity by releasing molecules which (1) render blood vessels permeable to plasma proteins; (2) coagulate extravasated fibrinogen to fibrin; and (3) remodel deposited fibrin by activating the fibrinolytic system (Dvorak *et al.*, 1982; Cederholm-Williams, 1981). The cellular immune response may contribute to these processes. Fibrin deposits that have been planted in normal tissue have been found capable of inducing angiogenesis and desmoplasia. It has therefore been reasoned that the "tumor angiogenetic factor" of Folkman (1974a,b) may not be a single product unique to tumors but rather a series of mediators, closely linked to the physiological process of wound healing, which leads to deposition and modulation of tumor fibrin or fibronectin (Dvorak *et al.*, 1982). Tumor angiogenesis and desmoplasia may be regarded as specialized examples of wound healing, a process whose cardinal features include fibrin and fibronectin deposition, angiogenesis, and fibrous connective tissue (scar) formation.

Recruitment of cells of the host defense system to sites of tumor growth and invasion has also been found to limit tumor growth. Agents such as bacillus Calmette–Guérin (BCG) and *Corynebacterium parvum*, for instance, which are used in cancer immunotherapy, seem to exert their effect via their ability to elicit inflammatory reactions and recruitment of tumoricidal activated macrophages. Figure 11 illustrates pathophysiological findings of tumor stroma development at different stages of tumor development using the immunogenic diethylnitrosamine-induced line 1 hepatocarcinoma, a transplantable tumor of strain 2 guinea pigs (Dvorak *et al.*, 1982). Shortly after tumor transplanatation, the development of a fibrin–gel meshwork was observed which was followed by fibroblast invasion and collagen production. By day 8, a cellular antitumor immune response developed which involved lymphocytes, basophils, and monocytes forming prominent cuffs around venules at the periphery of the now fibrous connective tissue that enveloped tumor cell clumps. Macrophages were particularly prominent in peripheral zones of hemorrhage where they were engaged in pha-

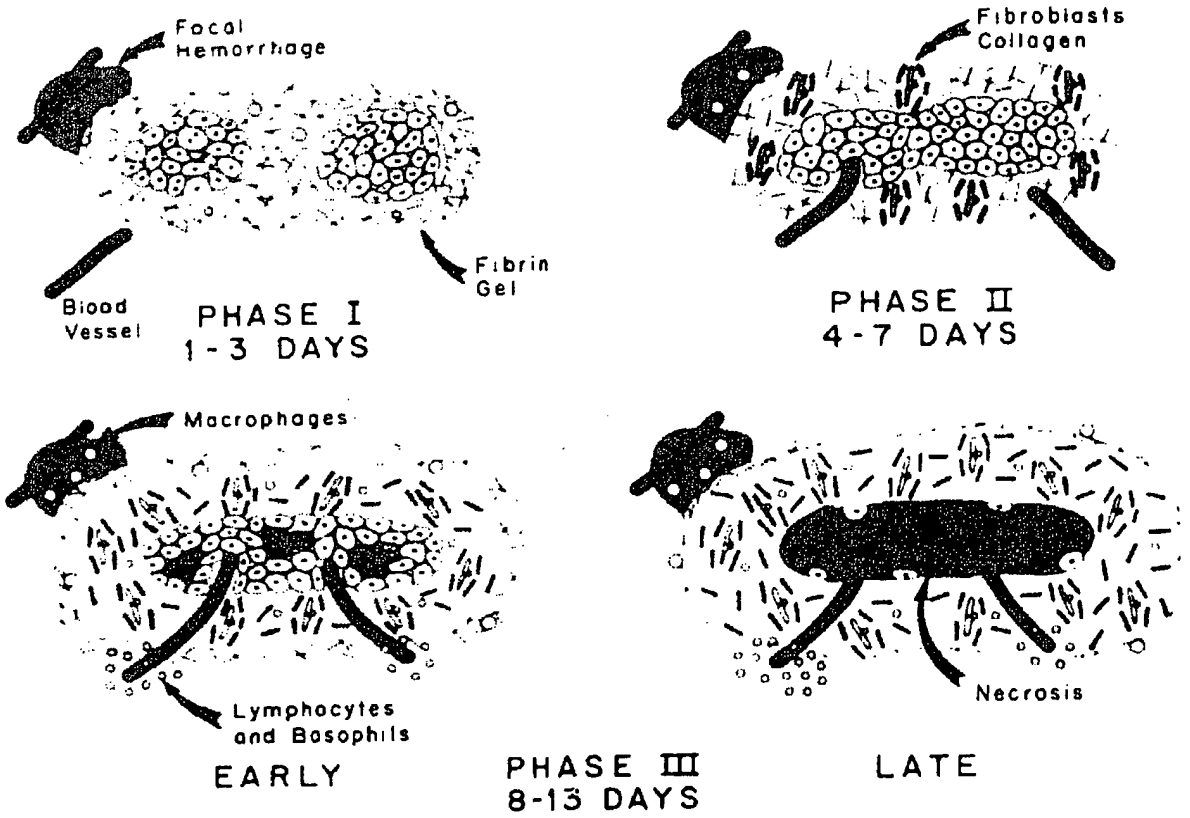


FIG. 11. Kinetics of tissue reactions toward a transplanted immunogenic tumor in the guinea pig showing early fibrous reaction and later immunological reactions eventually leading to tumor necrosis. (Reproduced from Dvorak *et al.*, 1982.)

gocytosing extravasated erythrocytes and debris. Extensive inflammatory cell invasion of the inner tumor cell mass was never observed. These tumors were apparently rejected by a mechanism involving extensive damage of the microvasculature which led to tumor infarction (Dvorak *et al.*, 1979a,b).

VII. Tumor Dormancy

The dormant tumor state has been defined as one in which tumor cells persist in a clinically normal host for a prolonged period under growth restraint, with little or no increase in the size of the tumor cell population (Wheelock *et al.*, 1981). There are numerous clinical reports that provide circumstantial evidence for the existence of dormant tumor states in man. One-third of the mortality from breast cancer, for instance, occurs more than 5 years after primary treatment (Adair *et al.*, 1974). One-fifth of patients with cutaneous melanoma who develop recurrence do so after an interval greater than 5 years (Holland and Frei, 1973). A primary melanoma and a metastatic melanoma occurring years later were shown to have common chromosomal

markers (Balaban *et al.*, 1982). By means of a monoclonal antiidiotypic antibody, small numbers of B lymphoma cells were identified in the circulation of patients during a clinical phase of remission (Hatzubai *et al.*, 1981). Both the preceding primary tumor and the succeeding secondary had the same idiotype.

Three principally different mechanisms of tumor dormancy have been distinguished in animal models: (1) avascularity and sequestration of tumor cells, (2) constitutive dependency of tumor cells on growth factors, and (3) immunologic restraint. The main reasons for mentioning tumor dormancy in the context of metastasis are (1) that this phenomenon illustrates that tumor–host interactions can reach a state of balance for prolonged periods, (2) that this can possibly be exploited to improve host control of tumor cells that survive cancer treatment, and (3) that it is important to find out what terminates a dormant tumor state thus abrogating the state of balance with the consequence of destruction of either the tumor or the host. Since these have been reviewed in detail elsewhere (Wheelock and Robinson, 1983), they shall be discussed only briefly here.

Small tumor nodules that do not develop a vascular network will be limited in size because of deficiency in nutrient diffusion. Such avascular tumor microspheres represent one type of tumor dormancy which has been demonstrated *in vitro* (Folkman and Hochberg, 1973) and *in vivo* (Gimbrone *et al.*, 1972). Sequestration of tumor cells within a capsule elaborated by the host can also inhibit tumor cell proliferation or invasion (Dvorak *et al.*, 1979a). A dormant tumor state can also be due to a constitutive dependency of tumor cells on growth factors, best known from hormone-dependent breast cancer (Noble and Hoover, 1975). The growth restraint will be broken, however, if tumor variants develop which elaborate their own growth factors and thus become autonomous (Yuhas and Tarleton, 1978). The first model of tumor dormancy by immunological restraint was demonstrated by Eccles and Alexander (1975). Fibrosarcoma cells that had metastasized to the lung prior to surgical excision of the primary tumor remained in the lung for many months in a dormant state. Immunosuppressive intervention, such as whole body irradiation or thoracic duct drainage, resulted in the outgrowth of lung metastases. The most intensively investigated animal model of tumor dormancy is probably that of Wheelock *et al.* (1982) who investigated the immunological restraint mechanisms of DBA/2 mice, which had been immunized ip with mitomycin C-treated syngeneic lymphoma cells (L5178Y) and subsequently challenged ip with viable L5178Y cells. Some mice remained clinically normal for many months but then suddenly developed ascitic tumors. It was found that a strong peritoneal cytotoxic T lymphocyte response was responsible for the establishment of the dormant tumor state. This then gradually waned but it could be reelicited *in vivo* or *in vitro* by reexposure to

tumor antigen. The maintenance of the dormant tumor state was ascribed to a synergistic cytolytic activity by peritoneal T lymphocytes and macrophages (Robinson and Wheelock, 1983). As the mouse proceeded through the dormant tumor state (2 months) and eventually developed an ascitic tumor, changes were observed in the tumor cells' phenotypic characteristics as well as in the host response (Wheelock *et al.*, 1982).

In our own studies with the L5178YE lymphoma (Eb), we found that a state of tumor dormancy could be established without preimmunization if the cells were inoculated into the hind footpads of syngeneic mice, a site where the tumor cells did not grow (Schirrmacher *et al.*, 1982a). When such tumor-inoculated mice were challenged with viable tumor cells sc in the back, a site where the tumor normally grows, no tumor growth was observed indicating that these animals had developed a status of antitumor immunity. However,in about 20% of thus challenged animals, an outgrowth of tumors was observed from their footpads. Termination of tumor dormancy in the footpad and tumor growth were also observed when animals previously inoculated with Eb tumor cells in the footpad were challenged unspecifically with allogeneic normal cells sc in the back (Schirrmacher *et al.*, 1982a). These findings indicate that dormancy due to immunological restraint can be "overcome" not only by immunosuppression (Eccles and Alexander, 1975) but also by immunostimulation (Schirrmacher *et al.*, 1982a). Immunologically controlled tumor dormancy obviously represents a delicate state of balance that is dependent on properties of the host (local microenvironment, status of the immune system) and on properties of the tumor cells (immunogenicity, metastatic capacity). The dependency of tumor dormancy on tumor cell properties was obvious from the fact that a spontaneous metastatic variant from the Eb tumor cells (ESb, Schirrmacher *et al.*, 1979), when inoculated into the footpad, behaved differently in that they grew out and metastasized.

VIII. Host Immune Responses in Metastasis

A. Spontaneous Regressions in Cancer Patients

Host control of tumor cell growth is best illustrated clinically in cases of complete disappearance of a histologically identified tumor in the absence of therapy. Such spontaneous regressions have intrigued clinicians for many years. Cases of spontaneous regression have been reported for most types of human cancer (Everson and Cole, 1966; National Cancer Institute Monograph, 1976). Overall, however, it seems a rare event. Sixty percent of documented cases have occurred in four types of cancer: malignant melano-

ma, hypernephroma, choriocarcinoma, and neuroblastoma. In some of the cases, tumor regression has been preceded by bacterial or viral infection, but in the majority this has not been the case. Malignant melanomas represent less than 1% of all cancers but account for 11% of reported cases of spontaneous regression. Approximately 40% of such patients with spontaneous regression were apparently cured with no recurrence on long-term follow-up. Regression or partial regression of cutaneous malignant melanoma was associated with a dense lymphocyte infiltrate (McGovern, 1975). After regression of the tumor, the lymphocytes disappear leaving behind scar tissue. In hypernephroma, cases have been reported of spontaneous regression of pulmonary metastases following nephrectomy for hypernephroma (Garfield and Kennedy, 1972). Such regressions appeared to be due to immunological rejection.

Spontaneous tumor regression has also been observed in animal tumors whether spontaneous in origin (Miller and Olsson, 1971), virus induced (Dietz *et al.*, 1977), or chemically induced (Rice, 1972). Such regressions were all mediated by immunological mechanisms. Nevertheless, there may be other mechanisms that could lead to spontaneous regression, for instance, induction of differentiation (as observed in neuroblastoma or in lymphomas) or deprivation of nutrients. Research on mechanisms of spontaneous regression of tumors, although difficult for logistical reasons, could lead to a better understanding of endogenous types of control of neoplastic growth and might lead to the development of ways to enhance these mechanisms for more effective cancer therapy.

B. Experimental Studies

1. Unspecific or Natural Immune Responses

A close association between levels of natural killer (NK) cell activity and the ability of the host to eliminate circulating tumor cell emboli has been reported (Hanna and Fidler, 1980; Hanna, 1982). This came out from studies in hosts with low NK activity (such as beige mice) or hosts with high NK activity (such as nude mice). Furthermore, adoptive cell transfer studies demonstrated that NK cells were effective *in vivo* in destroying circulating tumor cells before their extravasation, whereas they exerted only a minimal inhibiting effect on already established micrometastases (Hanna and Fidler, 1980). Adoptive transfer of a cloned cell line with NK activity markedly inhibited lung colony formation of B16 melanoma cells when inoculated intravenously into NK-deficient syngeneic mice (Warner and Dennert, 1982). In the same study it was shown that the incidence of radiation-induced thymic leukemia in C57BL/6 mice was dramatically reduced by iv

inoculation of cloned NK cells 3 months before the normal onset of leukemia. That NK cells may exert selective pressure on disseminated tumor cells during spontaneous metastasis is suggested from findings in several tumor systems indicating that tumor cells isolated from metastatic lesions showed a higher level of NK-cell resistance than tumor cells from the primary lesion (Fogel *et al.*, 1979; Gorelik *et al.*, 1979b). It has to be kept in mind, however, that not all malignant cells are readily susceptible to NK-cell-mediated lysis. Whether NK cells play a role in the elimination of circulating tumor cells in patients is not known at present.

Platelet aggregation and fibrin coating of the surface of tumor cells may be one of the mechanisms by which hematogenously spread tumor cells are protected from destruction by NK cells. Gorelik *et al.* (1984) recently showed that the antimetastatic effects of anticoagulant drugs, such as heparin and prostacyclin, depended on levels of NK activity in the host. It was therefore suggested that anticoagulant drugs may exert their antimetastatic effects by making tumor cells more vulnerable to NK-cell lysis rather than by blocking adherence of tumor cells to vascular endothelium.

Polymorphonuclear leukocytes could also contribute to natural defense reactions against metastases. Such cells were recently reported to play a role in the pulmonary clearance of arrested B16 melanoma cells (Glaves, 1983).

Natural antibodies appear to be important in host surveillance of at least some metastasizing tumors. It was shown by Vaage (1978) that normal mouse serum contains components, presumably natural antibodies and complement, which can kill mouse C3H mammary carcinoma and ovarian carcinoma cells *in vitro* as well as *in vivo* and which can inhibit metastasis formation. Similar findings were reported by Chow *et al.* (1981) from murine lymphoid tumor systems.

Another potentially important natural host defense system that could influence the outcome of metastasis is the mononuclear phagocyte system. The ease of monocyte mobilization to inflammatory sites, the widespread distribution of macrophages through the body, the exquisite synthetic machinery of the macrophage, and its potent antitumor activity imply that cells of this lineage could have a potentially important role in the control of neoplastic primary and metastatic growth. The mere presence of macrophages at the site of a growing tumor, however, does not appear sufficient for such a controlling function. The cells have to become appropriately activated to express tumor cytostatic and/or tumoricidal functions (Alexander, 1976; Hibbs, 1974; Evans, 1982; Key, 1983). Such activation can be achieved via priming and trigger signals delivered by nonspecific means, for instance by biological response modifiers (BRMs) or by T-cell-derived lymphokines, such as macrophage activating factor (MAF) (Meltzer, 1981). The most

promising new protocols for *in situ* activation of tumoricidal macrophages make use of liposomes containing either MAF or muramyl dipeptides (Fidler, 1980). Results from Fidler and Hanna (1981) indicate that the application of such liposomes, which were designed to home to the lungs and to activate *in situ* the lung macrophages, might become an important new tool in antimetastatic therapy. Control animals with a sc growing B16Bl6 tumor were all dead by 80 days, whereas 60% of the mice that had received a regimen of liposome-encapsulated muramyl dipeptide were still alive 170 days postimplantation.

It has been argued that tumor cell resistance to macrophage cytolysis is a rare phenomenon and that such variants if they exist at all, may arise with much lower frequency than phenotypes for resistance to other therapeutic modalities (Fogler *et al.*, 1980). Macrophage-resistant progressor tumor variants have, however, been described recently (Urban and Schreiber, 1983). It has to be kept in mind also that cytotoxic macrophages can become suppressed *in vivo* by T cells, immune complexes, or serum-blocking factors (Rao *et al.*, 1979; Hellström *et al.*, 1979). Furthermore, macrophages are themselves able to suppress immune T-cell functions (Ting and Rodrigues, 1980). In many animal systems, as well as in advanced cancer patients, there is evidence of impaired immunological functions, which may be related to such a kind of suppression.

2. *Specific Immune Responses and Tumor Immune Escape*

Spontaneous pulmonary metastases from an antigenic methylcholanthrene-induced murine sarcoma were reported by Sugarbaker and Cohen (1972) to have an altered antigenicity compared to that of the primary tumor. Similar findings of differences in immunogenicity and/or antigenicity between primary and secondary tumor lesions were later reported from various other systems (Pimm *et al.*, 1980; Fogel *et al.*, 1979; Gorelik *et al.*, 1979b; Schirrmacher *et al.*, 1979). These findings could be interpreted as the result of random antigenic variation occurring during tumor growth and host immune selection of distinct subpopulations occurring during metastasis. This would result in the outgrowth of tumor variants able to escape the specific immune response against the primary tumor.

Antigenic variation in cancer metastasis (Schirrmacher *et al.*, 1982c) as observed with chemically induced tumors may be possible because of the enormous polymorphism of tumor-associated transplantation antigens (TATA) which can become expressed on chemically transformed cells. Such TATAs have similarities to normal histocompatibility antigens not only with respect to polymorphism but also with respect to their ability to induce strong T-cell-mediated immune responses. Chemicals that are either muta-

genic (Boon, 1983), or that only affect DNA methylation (Kerbel and Frost, 1982) have been shown to change a tumor cell's immunogenicity as defined by immunization–protection assays.

The strong immunoselective pressure exerted by the host onto antigenic tumor cells during their process of metastasis could lead to an alteration in antigenicity, as discussed already, or it could lead to antigen loss and thus complete resistance to T-cell-mediated immunity. Such a process has been described and analyzed in detail in the ESb lymphoma model system (Bosslet and Schirrmacher, 1981, 1982). Twelve days after sc implantation of twice cloned TATA-positive ESb lymphoma cells that could be recognized and lysed by anti-TATA cytotoxic T lymphocytes (CTL), tumor variants could be detected from metastatic deposits in the spleen which were completely resistant to these syngeneic tumor specific CTLs. The variant cells were not generally resistant to CTL lysis because they could be killed by allogeneic anti-H2 CTL. Such selectively immunoresistant tumor variants arose with a high frequency under specific *in vivo* conditions. They were stable for over 100 cell generations in tissue culture and were not detectable in the parent cell population by either cloning or *in vitro* immunoselection procedures (Bosslet and Schirrmacher, 1982). A recent cytogenetic comparison between $TATA^+$ and $TATA^-$ ESb-type cells revealed distinct chromosomal differences (Dzarlieva *et al.*, 1982).

Antigen loss may occur only under certain circumstances when strong antigens elicit immune responses capable of eliminating antigenic cells at primary or secondary lesions. The same TATA-positive ESb cells as described above remained TATA positive when inoculated directly into the spleen (Bosslet and Schirrmacher, 1982) and also when grown in the peritoneal cavity. In fact, it is remarkable that the TATA has not been lost in our standard ESb cell line which has been transplanted ip for over 10 years since it first appeared in 1968. Antigen loss variants have also been described for the MDAY-D2 tumor (Dennis *et al.*, 1981c), but the frequency of occurrence during spontaneous metastasis from a sc site was lower than in the ESb tumor. The continued process of host selection, resulting eventually in the emergence of selectively immunoresistant variants, may also be a relatively common feature of slowly growing spontaneous neoplasms.

Since T-cell-mediated immune reactivities are restricted by gene products of the major histocompatibility complex (MHC), Feldman, Segal, and associates investigated whether tumor cell diversity in metastatic competence correlated with diversity in the expression of MHC gene products. Variations in MHC antigenic profiles of tumor cells have been frequently observed (Parmiani *et al.*, 1979) and their biological effects discussed (Festenstein and Schmidt, 1981). In the Lewis lung carcinoma, a correlation was found between the metastatic potential of subclones and an imbalance of

$H\text{-}2K^b/H\text{-}2D^b$ expression. The lower the ratio, the more metastatic were the clones (Eisenbach *et al.*, 1983). It is not yet resolved, exactly how *H*-2 gene products on tumor cells can influence metastatic capacity.

In addition to specific T cells there are specific antibodies that can influence metastasis formation. Shearman and Longenecker (1980) described a monoclonal antibody that could partially block liver colonization of chick Al-2 lymphoma cells. Expression of the corresponding antigen on the lymphoma cells correlated with enhanced liver colonization. Similar results were described in the RAW 117 lymphoma with antibodies against fetal determinants (Nicolson, 1982). Vollmers and Birchmeier (1983a) reported on a monoclonal antibody against B16 melanoma cells that could block plastic adhesion of the tumor cells *in vitro* and that inhibited lung colony formation *in vivo*. Such antibodies also interfered with adhesion of other tumor cell types including human carcinomas (Vollmers and Birchmeier, 1983b). Recent findings point toward an important role of tumor cell binding to laminin (L. A. Liotta and J. Varani, personal communications; Vollmers *et al.*, 1984) which may open new approaches to selective interference in the metastatic cascade process (see below).

New developments in hybridoma technology could lead to production of monoclonal antibodies that react preferentially with metastases of certain tumor types. Such antibodies may become of increasing importance for radioimmunodetection by radioimaging procedures (Farrands *et al.*, 1982) and perhaps also, when combined with drug targeting, for new therapeutic approaches. The present state of the art has recently been exemplified by studies on the *in vivo* localization of antihuman-osteogenic sarcoma monoclonal antibody in human tumor xenografts in nude mice (Baldwin and Pimm, 1983).

3. *Intratumoral Immunological Heterogeneity and Aspects of Immunoregulation*

Heterogeneity exists not only in host immune responses but also in the immunological properties of the tumor cells themselves. Tumors of the same histological origin differ from each other immunologically. Even within one and the same tumor there is immunological heterogeneity. This is reflected in variations of tumor-associated antigens, differentiation antigens, histocompatibility antigens, lectin-binding sites, and receptors for natural killer cells and natural antibodies (Heppner and Miller, 1983). Four antigenic subpopulations (A, B, C, and D) were, for instance, identified within a spontaneous AKR lymphoma (Olsson and Ebbesen, 1979). Treatment of tumor-bearing mice with a mixture of all four subpopulations was much more efficient in inducing protection than immunizations with mixtures lacking one or more of the subpopulations. Miller and Heppner (1979) described

antigenic heterogeneity of five tumor cell subpopulations derived from a single BALB/cf 3H mouse mammary tumor. Experiments in which mice were immunized with each subline separately or with mixtures thereof indicated that the specificities of the responses to the mixtures were not simply the sum of the responses to the individual sublines. Thus, the response to an immunogenic subpopulation may cause cross-protection of other subpopulations (Miller, 1982), whereas the response to a suppressogenic subpopulation could cause suppression of immune responses against other subpopulations.

Basic immunology has provided evidence that immune responses underlie controlling elements that can exert either positive or negative regulatory functions such as "helper" functions or "suppressor" functions. T-cell subpopulations with either helper or suppressor function could be distinguished among others by differences in expression of Lyt differentiation antigens. The existence of immunological regulatory circuits with suppressor and contrasuppressor systems has been proposed and described by Gershon and colleagues (Gershon *et al.*, 1981; Green *et al.*, 1983). Lymphocyte subpopulation interactions can be mediated by antigen-specific and -nonspecific soluble factors and corresponding cell surface receptors. They could also involve specific receptor–antireceptor interactions as suggested in Jerne's immunological network theory (Jerne, 1976). Such idiotype network interactions in antitumor immune reactions have been reviewed recently (Schreiber, 1984).

From these considerations it becomes apparent that the dynamics of tumor progression with continuously evolving subpopulations may initiate complex immunological reaction cascades that develop into a specific immunological microenvironment. Heppner and Miller (1983) used the term "specific ecosystem" to describe the complex tumor–host network interactions. It is not surprising from such a point of view that it is virtually impossible to generalize on the role of host immunity in malignant processes and in metastasis in particular. In some tumor systems suppression of antitumor immune responses resulted in increased metastasis, whereas in other tumor systems similar manipulations decreased metastasis formation. Using C3H fibrosarcoma cell lines of differing immunogenicity, Fidler *et al.* (1979) showed that weakly immunogenic fibrosarcoma cells grew and metastasized more readily in immunocompetent as compared to immunoincompetent hosts, whereas the reverse was true for intermediate immunogenic tumor cells, which is consistent with Prehn's idea of immune stimulation (Prehn, 1972).

One way in which metastatic cells may successfully evade host surveillance is by suppression of host immune responses. Fujimoto *et al.* (1978) found that suppressor activity could be transferred from tumor-bearing ani-

mals via spleen or thymus cells and could be abolished by anti-T-cell antibodies plus complement. Transfer of T suppressor cells resulted in the inhibition of the effector phase of T-cell-mediated immune cytolysis but only if the original sensitizing tumors were identical or very similar to the tumors being tested, indicating T suppressor specificity. Another interesting tumor system with regard to immunosuppression is that of UV light-induced skin tumors studied by Kripke and Fisher (1976). Chronic exposure of mice to UV light can produce fibrosarcomas and squamous cell carcinomas. These are highly immunogenic and are thus rejected after transplantation into syngeneic hosts. Pretreatment of such hosts, however, with intermittent doses of UV light could render them tolerant to the tumor transplants as if they were suppressed. The effect could be shown to be due to activation of T suppressor cells which prevented immune destruction of the highly antigenic tumors (Fisher and Kripke, 1978). In contrast to antitumor cytotoxic T lymphocytes (CTL) which could specifically distinguish individual antigenic determinants on different UV-induced tumors (Wortzel *et al.*, 1982), the T suppressor cells seemed to recognize cross-reactive determinants which are apparently shared by all the UV-induced tumors but which are not expressed on chemically induced tumors (Urban *et al.*, 1982).

Naor (1983) has recently summarized findings from different tumor systems which suggest the coexistence of suppressogenic and immunogenic determinants within a tumor. Such determinants could be expressed on the same cell or on different tumor cell subpopulations. More refined technologies are required on the cellular and molecular level to unravel the nature of such determinants with opposite immunological effects. If the findings can be substantiated, however, they may become very important. It would then become quite rewarding to either eliminate the suppressogenic determinants or to alter them in such a way that they lose their suppressogenic capacity and perhaps acquire immunogenic capacity instead.

In a recent report (Cianciolo *et al.*, 1983), suppressogenic molecules have been identified on murine malignant but not on normal cells as a 19-kDa protein being antigenically related to the immunosuppressive retroviral protein, p15E.

IX. Impacts of Experimental Studies on Cancer Treatment Strategies

A. New Strategies for Immunological Intervention

The search for an immunological approach to the treatment of cancer derives as a logical extension of the effectiveness of the immune response in dealing with infectious diseases. Theoretically, the immune system should

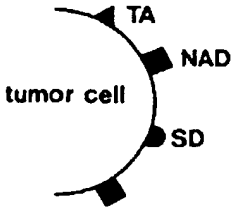
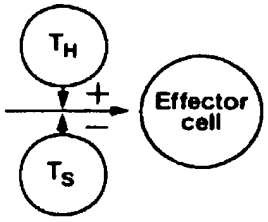
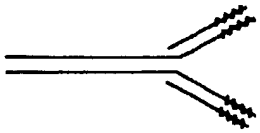
be particularly effective against disseminated diseases and micrometastases as opposed to primary tumors because of the different numbers of tumor cells to be eradicated and the more favorable ratio of effector to target cells when dealing with small tumor foci. As discussed more extensively elsewhere (Frost and Kerbel, 1983), cycles of euphoria with regard to tumor immunotherapy have, to date, generally been followed by unsuccessful clinical trials. This was felt not to be surprising, however, when considering the diverse ways in which a host can respond to its tumor cells, the problem of tumor heterogeneity and progression and of subpopulation interactions, the weak immunogenicity of many "spontaneous" tumors, and the number of different escape routes available to highly malignant cells (Kim, 1979). Another reason for the discrepancy between the experimental and clinical data could be that most of the experimental animal tumor systems in which immunotherapy protocols proved to be effective may not have mimicked closely enough the human cancer and the course of its disease development (Eccles, 1982).

Nevertheless, the possiblities of immunological intervention in metastasis should be exploited systematically. This should be done in appropriate experimental model systems first and should not be too hastily applied to the clinical situation as long as there is only a poorly defined immunological basis. While immunologic thinking has shifted in recent years toward the development of mainly unspecific immune stimulation modalities, at least some investigators feel that specific immunological intervention is still feasible and should not be forgotten. This hope is based on recent advances in our understanding (1) of the relationship of the immune response to metastasis, (2) of new ways to increase the immunogenicity of spontaneous tumors, (3) of insights into immunoregulatory phenomena as well as (4) of experimental antimetastatic-specific immunotherapy protocols, which already have proved to be effective.

Some experimental approaches for immunological intervention in metastasis are listed in Table XI. They include efforts (1) to increase the tumor's immunogenicity, (2) to interfere with host immune regulatory mechanisms, (3) to restore or increase immune competence, for instance, by adoptive cell transfer, and (4) to exploit monoclonal antibodies for interference at specific steps of the metastatic process. Both unspecific and specific components of the host defense system should be tested separately or in combination in order to design new strategies for immunotherapy of metastases.

An increase in tumor immunogenicity can be obtained by various means, such as chemical modification, virus infection, treatment with mutagens, or somatic cell hybridization. Immunization against such modified tumor cells often resulted in the immune rejection of the nonmodified tumor cells. Protective immunity against an apparently nonimmunogenic tumor line has

TABLE XI
IMMUNOLOGICAL INTERVENTION IN METASTASIS: EXPERIMENTAL APPROACHES

Approach[a]		References
Increase in tumor immunogenicity		
	Chemically modified cells Mutagenized tumor variants Virus-infected tumor cells Somatic cell hybrids	Hamaoka *et al.* (1979), Prager and Baechtel (1973), Boon (1983), Kobayashi (1979), Austin and Boone (1979), Kawashima *et al.* (1983)
Interference with immunoregulation		
	Elimination of suppressor cells Immune stimulation unspecific unselective Immune stimulator specific selective	Dye and North (1981), Naor (1979, 1983), Greene *et al.* (1977), Hanna *et al.* (1979), Hanna and Key (1982), Fidler (1980)
Adoptive cell transfer		
	NK cells Syngeneic immune T cells Allogeneic H-2 identical immune T cells Il-2 expanded CTLs	Hanna and Fidler (1980), Warner and Dennert (1982), Treves *et al.* (1975), Schirrmacher *et al.* (1982), Frost and Kerbel (1983), Wiltrout *et al.* (1979), Schirrmacher (1979), Dailey *et al.* (1982), Eberlein *et al.* (1982), Kedar and Weiss (1983)
Inhibition by monoclonal antibodies		Vollmers and Birchmeier (1983)
		

[a] TA, Tumor antigen; NAD, new antigenic determinant; SD, suppressogenic determinant; T_H, T helper cells; T_S, T suppressor cells.

been observed in some tumor systems (Boon, 1983; Kobayashi, 1979; Kawashima *et al.*, 1983). If such one-way cross-reactivities and possibly subpopulation interactions could be created and/or selectively enhanced, active immunotherapy might be beneficial even against nonimmunogenic cells. This might involve the isolation or activation of a "controlling" subpopulation by treatment with mutagens, manipulation of major histocompatibility antigen expression, infection with a virus, or chemical modification (Table XI). Efforts to interfere with immunoregulation might be directed toward the elimination of suppressor cells and/or toward stimulation of immune effector mechanisms. This may involve the use of irradiation regiments, drugs, and biological response modifiers. Such interference with immunoregulation should be as selective as possible and should have minimal side effects. One example of a selective interference is the use of macrophage activating liposomes that are designed to home to particular organs.

A direct approach for specific immunotherapy of metastases was assessed by Treves *et al.* (1975) by adoptive transfer into tumor-bearing animals of T cells sensitized to irradiated tumor cells *in vitro*. The treatment was only partially successful, probably because of the interference of T suppressor cells. That T supressor cells generated in tumor-bearing mice could impair the effectiveness of adoptive immunotherapy has indeed been demonstrated (Dye and North, 1981). Later, a potent ability of T cells to inhibit metastases was demonstrated independently by two groups, both of which used allogeneic but H-2-identical T cells in adoptive immunotherapy protocols. Wiltrout *et al.* (1979) demonstrated that the adoptive transfer of spleen cells from immunoresistant BALB/c *nu*/+ tumor immune animals into BALB/c *nu/nu* mice bearing the metastatic DBA/2 tumor MDAY-D2 could cause arrest and reversal of established visceral metastases. In a similar but syngeneic system, the author (Schirrmacher, 1979) could show that DBA/2 mice bearing the highly metastatic DBA/2 tumor ESb could be partially protected from death by metastases by the iv transfer of spleen cells from B10.D2 tumor immune animals. A single cell transfer led to a 100% increase of life expectancy. Recently Frost and Kerbel (1983) modified their immunotherapy protocol for application in a syngeneic system: First they used antitumor CTLs which were raised against an immunogenic variant of MDAY-D2 obtained by mutagen treatment. This variant did not induce suppressor cells and generated a much higher antitumor CTL response. Before adoptive cell transfer into syngeneic MDAY-D2 tumor-bearing mice, the primary tumor was surgically removed and eventual host suppressor cells (Bursuker and North, 1984) eliminated by irradiation. With this protocol, a large percentage of animals bearing metastases (about 75%) were cured, and the remaining 25% showed prolonged survival as compared to controls. Similar effective immunotherapy results were recently obtained by our group in the ESb

tumor system, using a virus xenogenization approach (unpublished findings). Many parameters for an optimal and highly reproducible response in such an experimental immunotherapy protocol still have to be worked out. Furthermore, the mechanisms underlying such an effective protective immunity need to be unraveled in order to know in which direction to go if such protocols are to be tested for clinical applicability.

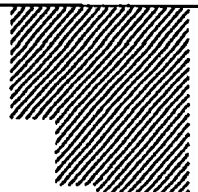
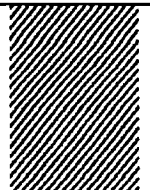
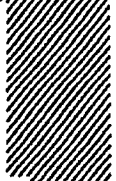
In both highly metastatic tumor models, MDAY-D2 and ESb, effective protection against metastases could thus be obtained in spite of the fact that the tumors used are poorly immunogenic, are heterogeneous, and have a strong tendency to develop tumor antigen loss variants. It is possible that in both systems the specific cellular antitumor immune responses were flanked by nonspecific cytotoxic mechanisms mediated by macrophages and possibly NK cells. There are additional possibilities for further improvements of the therapy protocols: (1) the use of Il-2 and of Il-2 expanded long-term T-cell lines (Donohue *et al.*, 1984), (2) a combination of adoptive specific immunotherapy with macrophage activation via liposomes containing activating agents, and (3) combinations of immunotherapy with other treatment modalities. Synergistic effects of active specific immunotherapy and chemotherapy have been reported (Key *et al.*, 1983). It appears that immunotherapy, if designed properly, still has a great potential. Antimetastatic immune T-cell therapy, although not yet practical, would be a means of achieving systemic, nontoxic, and nonmutagenic therapy. The potential role of T cells in cancer therapy has recently been reviewed (Fefer and Goldstein, 1982; Cheever *et al.*, 1983).

B. Other Impacts from Experimental Studies

The sequential and stepwise evolution of metastasis has a number of implications for treatment strategies. In addition to direct anticancer drugs there could be strategies developed to intervene at any one critical point during the multistep process of the metastatic cascade. Some examples, as derived from experimental studies, are given in Table XII. The treatment strategies listed aim at specific therapy targets which evolve from the analysis of mechanisms at each successive step of disease progression (e.g., angiogenesis, invasion, clumping, capillary adhesion, extravasation). Each step in the cascade is supposed to involve different tumor cell subpopulations and different mechanisms of tumor–host interactions. Such steps are illustrated in the left part of the table. References are given to the sections in this article where respective details have been discussed. The table is not meant to be complete but rather to illustrate possibilities of rational approaches to therapeutic intervention in cancer metastasis.

Combinations of drugs may be found to prevent angiogenesis as ex-

TABLE XII

Metastatic cascade[a]				Treatment strategy		
Event	Tumorigenic noninvasive subpopulation	Invasive nonmetastatic subpopulation	Metastatic subpopulation	Therapy target	Method[b]	Reference section
1. Uncontrolled proliferation				Activation of fibrous "barrier" formation	?	VI
2. Angiogenesis and local invasion				Prevention of angiogenesis Antiinvasive treatment	Use of combined drugs and AIF	V VI
3. Survival in the circulation				Prevention of clumping Stimulation of NK activity	Anticoagulant drugs BRM	VIII
4. Arrest and extravasion				Inhibition of adhesion	Monoclonal antibody, laminin fragment	II
				Stimulation of M ϕ activity	Liposome-MAF	VIII
5. Proliferation at new site into secondary foci				Antiproliferative antitumor agents	Chemotherapy Radiotherapy	
				Systemic activation and restoration of immune capacity	Immunotherapy	V
6. Evasion of host defense and resistance to therapy				New subpopulation diversification with emergence of resistant variants	Change treatment regiments in rapid succession	IV
7. Tumor dormancy				Prevention of "activation" of dormancy	?	VII
8. Angiogenesis and local invasion				Prevention of angiogenesis Antiinvasive treatment	Systemic application of selected drugs (see Step 2)	V VI
9. 3° metastases from 2° metastases				Steps 3–7		III

[a] Modified version from that of Poste (1982).

[b] BRM, Biological response modifiers, MAF, macrophage activating factor.

emplified by Folkman's recent quite successful experimental studies (Folkmann *et al.*, 1983). This could have an inhibitory effect not only in step 2 but also in step 8, which initiates the development of metastases from metastases. Cancer invasion may be prevented by antiinvasive drugs (Mareel, 1984) or antiinvasive tissue factors (AIF; Eisenstein *et al.*, 1975). Although cancer invasion has to be considered an important target for therapy because this step leads to progression from a benign to a malignant state, the way that screening of anticancer drugs has been conducted so far has neglected this aspect and still does. In colon cancer, depth of invasion (Duke's classification) is an important prognostic factor. The 5-year survival rate drops from about 70% for tumors that are limited to the mucosa to about 10% for tumors that have invaded all layers of the colonic wall. Potential inhibitors of invasion are ICRF-159 [1,2-bis(3,5 dioxopiperazin-1-yl)propan] (which inhibits intravasation in some experimental tumors), protease inhibitors such as aprotinin, or microtubule inhibitors such as vindesine or podophyllotoxin (Mareel, 1982). The ideal antiinvasive agent should prevent invasion and transform a malignant into a quasi benign tumor without the dose-limiting toxicity of current antiproliferative or cytotoxic drugs. Such an ideal antiinvasive agent with high selectivity has not, however, yet been found.

Agents acting on the coagulative–anticoagulative equilibrium and at the fibrin system (Maat and Hilgard, 1981) could influence particular steps such as activation of a fibrous barrier, prevention of invasion or of tumor cell clumping, and capillary arrest in the circulation. Most experimental evidence for the antimetastatic effect of anticoagulants came from experimental metastasis assays after intravenous tumor cell inoculation. Coumarin and derivatives thereof produced a constant antimetastatic effect that was independent of the investigator and of the tumor model. Although antimetastatic effects of anticoagulants have failed so far in spontaneous metastasis assays, interest in coumarins as potential antiinvasive agents is supported by recent findings that warfarin treatment significantly retarded disease progression in small cell carcinoma of the lung (Zacharski *et al.*, 1981).

Specific reagents that can inhibit the organ implantation phase should also be looked for and developed. Blocking of distinct cellular receptors such as the receptor for laminin and use of selected monoclonal antibodies are two examples discussed in Section VIII. Immunological means of interference with metastases have been summarized and discussed in Section IX,A.

Because of the problem of phenotypic instability of tumor cell subpopulations that survive a particular therapeutic treatment, the object of new strategies should be to prevent new diversification and outgrowth of resistant variants. Therefore it seems advisable to change treatment regimens in rapid succession and to use combinations of treatments that aim at different

therapeutic targets and that make use of as many as possible modalities of treatment. The involvement of the body's own defense systems, both natural and specific, should always be a part of the overall strategy because this can interfere in many different ways and in many different steps.

Genetic instability and tumor subpopulation interactions (discussed in Section IV) could have profound impacts on treatment strategies. Most cancer treatments, if not complete, might influence phenotypic diversity among the remaining tumor cell subpopulations and might thereby affect tumor progression. Individual tumor cell subpopulations may differ significantly in their sensitivities to various therapeutic agents and this in itself represents a major obstacle to the development of new therapeutic strategies of cancer treatment. Advances in our knowledge on the complexities of tumor cell heterogeneity and diversity (Section IV) have rendered many of the traditional therapeutic strategies obsolete. Screening programs for potential anticancer agents have to take into account more than before the problems of phenotypic heterogeneity, the possible impact of the drugs themselves on the generation of instability and diversification, and the side effects on normal host tissues and on the immune system which again could influence metastatic progression. Obviously, the most successful therapies will be those that circumvent phenotypic diversity among tumor cells and those that alleviate the problem of survival of resistant subpopulations during therapy.

The timing of therapy is also an important parameter because the cascade theory (Section III) suggests that the metastatic process develops sequentially by stepwise progression in time and space. There may be certain periods where antiproliferative anticancer agents can be expected to work most efficiently because a high proportion of cancer cells would be in a proliferative state. The removal of a large primary tumor mass, for instance, can be expected to lead to the removal of antiproliferative and other regulatory factors (Section III,B) which could stimulate the outgrowth of micrometastases. The application of antiproliferative anticancer drugs in this phase (Table XII,5) would thus be indicated.

Some experimental studies indicate that certain common procedures in the clinic might have a stimulatory effect on metastases. Examples are reported where the use of certain anesthetic drugs (Shapiro *et al.*, 1981) or of certain surgical procedures (Gorelik *et al.*, 1979a; Keller, 1984) caused acceleration of the progression of postoperative metastases. Immunosuppressive effects have been documented for anesthetics and surgery (Walton, 1978; Lee, 1977) as well as for chemotherapeutic agents. More effort should be devoted to the development of methods to prevent or counteract the possible negative side effects that might be inherent in many of the present clinical treatment procedures.

X. Summary and Conclusions

It has been the purpose of this article to describe recent advances in cancer metastasis research. Clinical realities and experimental approaches to the study of underlying basic mechanisms of metastasis formation were discussed. Wherever possible, results were reported which led to the development of theoretical concepts. Such results and concepts were finally evaluated in light of their possible impact for the design of new treatment strategies. Experimental findings from many diverse research fields were summarized with the help of tables, figures, and references.

It was concluded that the process of metastasis is a dynamic event that can be described as a sequence of interrelated steps. Experimental results indicated that malignant cells that migrate and disseminate from the primary organ to distant sites and there eventually develop into metastases have to survive a series of potentially lethal interactions. Intimate tumor–host interactions were reported to take place all along the metastatic process. They were elucidated at the steps of angiogenesis, invasion, organ interaction, dormancy, tumor rejection, and tumor immune escape. The outcome of such tumor–host interactions seemed to depend on intrinsic properties of the tumor cells themselves as well as on the responsiveness of the host.

Metastasis does not appear as a merely random process. Both clinical and experimental studies revealed that the whole process can be described more appropriately in terms of stochastic, sequential, and selective events, each of which is controlled and influenced by a number of mechanisms. With regard to therapeutic intervention, a selective event offers more possibilities than a random one because it is governed by rules that can be exploited experimentally. Various impacts from experimental studies for the design of antimetastatic cancer treatment strategies were discussed. Sequential steps of the metastatic cascade could become new therapy targets. Conventional empirically derived treatment modalities should become flanked by methods aimed more specifically at critical steps of cancer spread in order to prevent progression of the disease. This is where basic research on mechanisms could make significant contributions to therapy planning in the future. Furthermore, possible negative effects of surgery, radiotherapy, and adjuvant chemotherapy or immunotherapy that could result in enhancement of metastatic progression need to be critically evaluated to limit them as much as possible.

The most formidable obstacle to the successful treatment of disseminated cancer may be the fact that the cells of a tumor are biologically heterogeneous and can generate phenotypic diversity even if they arose from a single clone. Genetic instability of malignant cells, subpopulation interac-

tions, and host selection seem to be important keys to understanding the evolution of tumor heterogeneity and progression. Experimental metastasis research could have a significant impact on the design of clinical treatment regimens if it were able to unravel the mechanisms controlling the generation of phenotypic diversity and progression. The potential danger that chemotherapeutic drugs themselves could facilitate tumor progression via generation of "mutants" or DNA methylation variants (Boehm and Drahovsky, 1983) could then possibly be limited. Screening of potential anticancer drugs for their abilities to inhibit the growth of tumors containing widely heterogeneous subpopulations of metastatic and nonmetastatic cells may not be sufficient to predict the efficacy against metastases. New methodologies and more specific antimetastatic screening procedures are therefore urgently needed.

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